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# Physical properties of autoclaved bone

## Torsion test of rabbit diaphyseal bone

Structural properties of autoclaved diaphyseal bone in the rabbit were investigated by torsional test. Heat propagation into the bone was studied by means of thermocouples. The torsional test included 54 pairs of diaphyseal bones. Autoclaving was performed to the same degree of sterilization, although with variations of time and temperature. Standard autoclaving at 121°C for 20 min was found to cause a moderate decrease (23 per cent) in torsional strength. The decrease was more pronounced (35 per cent) for bones autoclaved at 110°C for 255 min and less (9 per cent) for those autoclaved at 131°C for 2 min. Heat propagation into bone during autoclaving proved to be rapid at both 121°C and 131°C, indicating that complete, uniform sterilization of diaphyseal bone may be performed to an accurate, predetermined degree. Diaphyseal bone subjected to standard autoclaving remains mechanically adequate for skeletal substitution. Reimplantation of autoclaved tumorous bone might provide a simple combined means for tumor devitalization and subsequent reconstruction.

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Reconstruction of skeletal defects sometimes present difficulties for the orthopedic surgeon. It has been reported that bone with a pathologic lesion (tumor, infection, ect.) may be resected and reimplanted after autoclaving, providing a simple combined means for devitalization of the specimen and subsequent reconstruction (Orell 1934, 1937, Thompson & Staggal 1956, Smith & Simon 1975, Johnston et al. 1983). However, conclusive data are still lacking with respect to the structural properties of bone after autoclaving and the completeness of bone devitalization by autoclaving.

We have studied structural properties of diaphyseal bone after autoclaving and propagation of devitalizing heat into diaphyseal bone during autoclaving.

## Material and methods

Fifty-four pairs of diaphyseal bones (humerus, femur, and tibia) were collected immediately after killing (I.V. pentobarbital sodium) 30 adult rabbits. The specimens were placed in plastic bags and kept at −70°C for 3 to 80 days. The bones were thawed at room temperature in saline-moistened gauze for 20 hours and were subsequently freed from the soft tissues. The autoclave (GE, 606 AR-1, Getingeverken, Getinge, Sweden), which permitted alterations of time and temperature, was equipped with thermocouples for continous monitoring of the temperature in the autoclave and autoclaved objects.

The degree of sterilization (F0) obtained by autoclaving is a function of time (t) and temperature (T): $F0 = t \times 10^{T-121 \times 10^{-1}}$ The degree of sterilization applied in clinical practice for easily damaged goods, such as rubber, etc., corresponds to an F0-value of 20. Therefore, we chose F0 = 20, although with variations of time and temperature. Then femora were autoclaved at 110°C for 225 min, 10 femora, 10 tibiae, and 14 humeri at 121°C for 20 min and 10 femora at 131°C for 2 min. The contralateral bone of each pair served as an unautoclaved control.

To monitor heat propagation into bone during autoclaving, thermocouples with a diameter of 2.5 mm were inserted in the medullary canal (Figure 1) of 16 femora through drill holes in the distal end (8 femora at 121°C and 8 femora at 131°C).

The structural properties of the specimens were studied by means of a computerized torsion test machine (Strömberg 1975, Johnsson & Strömberg 1985). After firm fixation of both ends, all 108 bones were twisted inwards at a constant speed (6 degrees/sec) until fracture. Simultaneously, the torque twist relationship was graphically recorded. From each curve, strength (maximum torque capacity), stiffness (linear slope), linear deformation (linear phase),

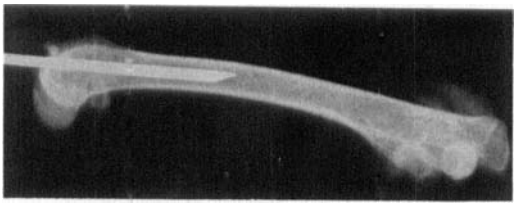

Figure 1. Thermocouple inserted into the medullary canal of a rabbit femur.

and total deformation (angle of fracture) could be derived (Figure 2).

The ratio between autoclaved and non autoclaved bone with respect to the recorded variables were calculated. The two-tailed Student's *t*-test was used for the statistical analysis. *P*-values > 0.05 were considered non-significant.

## Results

The pairs had to be excluded because of inadequate casting, which could be derived from the curves showing an atypical torque-twist relationship, and two pairs, because of computer processing errors.

The autoclaved bones showed a definite decrease in strength, stiffness, and weight when compared with the contralateral nonautoclaved bones, whereas the deformation changes were less distinct. The angle of fracture proved to be the only variable showing an increase after autoclaving, though not consistently.

The decrease in strength and stiffness was most pronounced for specimens autoclaved at low temperature (110°C) for a long time (225 min) and least pronounced for specimens autoclaved at high temperature (131°C) for a short period (2 min) (Table 1). The loss of strength and stiffness was greater ($p < 0.05$) for the low temperature – long time group (110°C/225 min) than for the group autoclaved at 121°C for 20 min. The loss of strength and stiffness was found to be greater ($p < 0.001$) for specimens autoclaved at 121°C for 20 min than for those autoclaved at 131°C for 2 min. Thus, there appeared to be a definite relationship between heat exposure and changes in structural

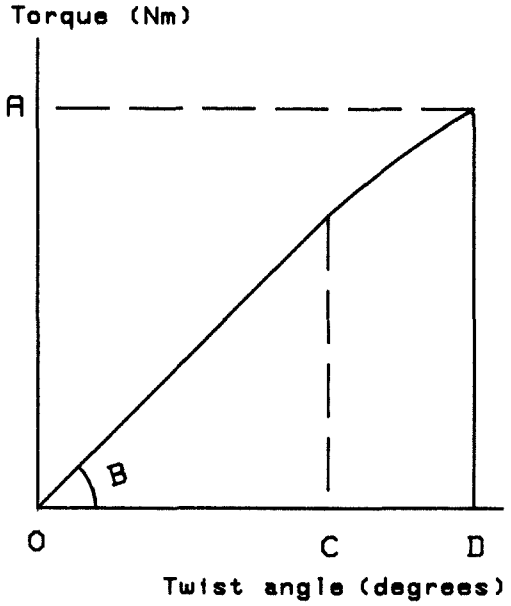


Figure 2. Schematic bone torque-twist diagram: O-A (Nm) = maximum torque capacity = strength, B (degrees) = linear slope (torque-twist relationship) = stiffness, O-C (degrees) = linear phase = linear deformation and O-D (degrees) = angle of fracture = total deformation.

*Table 1. Structural properties of autoclaved bone*

| FO 20 Groups Temp. (C) | Time (min) | n | Ratio autoclaved/nonautoclaved bone[a] Strength | Stiffness | Deformation Linear | Deformation Total | Weight |
|---|---|---|---|---|---|---|---|
| 110 | 255 | 7 | 0.65±0.07[b] | 0.73±0.08[c] | 0.74±0.11[d] | 0.97±0.07[ns] | 0.88±0.04[d] |
| 121 | 20 | 9 | 0.77±0.07[c] | 0.80±0.03[b] | 0.86±0.19[ns] | 1.05±0.14[ns] | 0.95±0.05[d] |
| 131 | 2 | 9 | 0.91±0.06[c] | 0.90±0.04[d] | 0.85±0.15[ns] | 1.05±0.09[ns] | 0.92±0.04[d] |

[a]Mean ± standard deviation.
[b]$p<0.001$.
[c]$p<0.01$.
[d]$p<0.05$.
[ns]= nonsignificant.

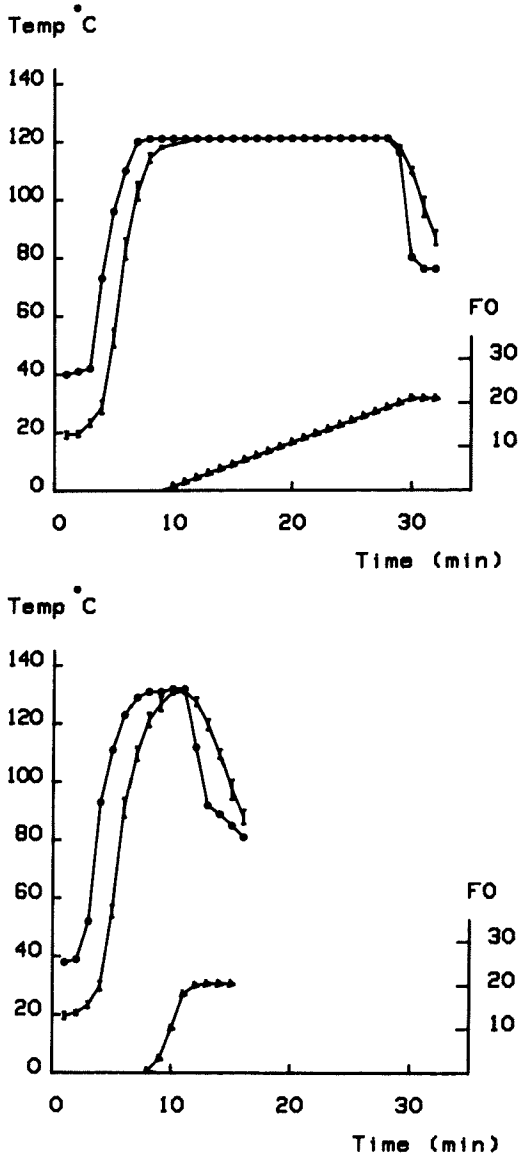


Figure 3. Temperature in relation to time for both autoclave (●) and autoclaved bone (+) at 121°C (top) and 131°C (bottom), both procedures providing the same degree of sterilization (FO = 20). The FO-value in relation to time is denoted by triangles (▲).

properties of bone, although the degree of sterilization (FO = 20) was the same.

As expected, the autoclaved (121°C/20 min) bones of different size and form (humerus, tibia, femur) consistently showed a decrease in strength (range 0.80–0.77), stiffness (range 0.80–0.76) and weight (range 0.95–0.94). However, no difference in changes of structural properties could be demonstrated between these different bones.

Heat propagation into bone during autoclaving was rapid (Figure 3). The time of heat propagation into bone was almost the same at 121°C and 131°C. However, at the higher temperature, the time needed for reaching the aimed temperature (131°C) in the bones comprised a larger proportion of the whole autoclaving time. Consequently, these bones were exposed to optimum heat for a very short (2 min) period of the whole autoclaving time.

## Discussion

Our study shows that autoclaving causes a moderate decrease in strength, stiffness, and weight of diaphyseal bone. Further, heat propagation into diaphyseal bone during auctoclaving is very rapid, indicating that the method can be used safely for uniform and complete sterilization (devitalization) of entire specimens to an accurate, predetermined degree.

Previous studies have shown that the *non-linear* phase of a torque twist diagram reflects microcracking in cortical bone (Netz 1979), probably in the hydroxyapatite prior to ultimate fracture. The changes in the *linear* phase as now demonstrated in diaphyseal bone after autoclaving are more likely to apply to collagen, which is supposed to undergo denaturation and degradation when heated above 60°C (Neustadt 1963). The observed decreased in weight of bone after autoclaving can probably also be related to collagen, which is known to pass into solution as gelatin when bone is exposed to moist heat (Burwell 1969). Loss of water seems less likely, since autoclaving was performed in saturated steam.

The current study differs in several respects from other studies on bone exposed to heat. Thus, Amprino (1958) studied the effect of dry heat on small pieces of cortical bone by the Brinell method and found increasing microhardness from 37°C to 120°C followed by a distinct decrease. Sedlin (1956) investigated bending strength of boiled pieces of human cortical bone, but could not with certainty demonstrate any changes. The difference between our results and other studies should mainly be attri-

buted to different types of heat exposure. Furthermore, other studies exclusively dealt with mechanical properties (pieces of bone), whereas the present investigation took structural properties (whole bones) into account.

In this context it seems appropriate to point out that the effect of deep freezing and thawing is small (Strömberg & Dalén 1976). Moreover, it does not influence a comparative analysis of autoclaved and nonautoclaved bone, otherwise treated uniformly.

We found a clear relation between autoclaving time and temperature, on the one hand, and changes in structural properties of bone, on the other, although the degree of sterilization (F0 = 20) was kept constant. Thus, within a certain range, it appears possible to optimize autoclaving time and temperature with respect to structural properties of bone without compromising the degree of sterilization. Because the least reduction in torsional strength (9 per cent) was noted for specimens autoclaved at 131°C for 2 min, this would seem to be the method of choice. However, the procedure entails a very short, and thus critical, period of optimal heat, which may be difficult to manage and control. From the point of view of devitalization, it appears safer to expose bone to a temperature (>100°C), which beyond doubt eventually causes cell death, for a longer period of time than only 2 min. In fact, it is unclear what degree of *sterilization* provides complete *devitalization* of whole bone specimens. Apart from this important aspect, practical reasons also speak in favor of autoclaving bone at 121°C for 20 min, since most standard surgical autoclaves are adjusted accordingly.

The observed decrease in torsional strength (23 per cent) of diaphyseal bone after standard autoclaving may be considered moderate, in as much as it has been reported that bone affected by a 50 per cent reduction of its maximum strength still can resist normal loads (Låftman et al. 1980).

By appropriate adjustment of autoclaving time and temperature, the physical effects on bone may be minimized within limits consistent with normal mechanical loads without compromising complete devitalization. Hence, resected pathologic bone may be used as a graft after autoclaving, provided incorporation can be attained.

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