There have been conflicting reports on the effects of gamma irradiation on the material properties of cortical allograft bone. To investigate changes which result from the method of preparation, test samples must be produced with similar mechanical properties to minimise variations other than those resulting from treatment.

We describe a new method for the comparative measurement of bone strength using standard bone samples. We used 233 samples from six cadavers to study the effects of irradiation at a standard dose (28 kGy) alone and combined with deep freezing. We also investigated the effects of varying the dose from 6.8 to 60 kGy (n = 132).

None of the treatments had any effect on the elastic behaviour of the samples, but there was a reduction in strength to 64% of control values (p < 0.01) after irradiation with 28 kGy. There was also a dose-dependent reduction in strength and in the ability of the samples to absorb work before failure.

We suggest that irradiation may cause an alteration in the bone matrix of allograft bone, but provided it is used in situations in which loading is within its elastic region, then failure should not occur.

Received 24 March 1995; Accepted after revision 1 September 1995

The use of large load-bearing allografts for the management of severe bone loss in revision arthroplasty is increasing (Allan et al 1991; Zmolek and Dorr 1993; Bridle and Oakeshott 1994; Chandler et al 1994; Henry, Reilly and Brick 1994). There is concern that fresh-frozen graft tissue may be able to transmit viruses, particularly human immuno-deficiency virus (HIV) and hepatitis viruses B and C (Nemzek, Arnoczky and Swenson 1994). HIV has been shown to reside in bone itself as well as in blood or marrow elements (Buck et al 1990). Transmission of HIV has been reported from a donor who was seronegative at the time of death (Simonds et al 1992).

In addition, femoral heads removed at hip replacement or cadaver bone taken with maximum sterility may become contaminated. Ivory and Thomas (1993) reported their experience of the Leicester bone bank over 18 months, where four of 22 large grafts (18%) from cadaver donors were contaminated. Of 91 bones taken in sterile conditions for the Massachusetts General Hospital bone bank only 18 had negative bacteriological cultures (Doppelt et al 1981).

High infection rates in fresh-frozen allografts may prompt the introduction of secondary sterilisation (Loty et al 1990) and gamma irradiation is a well-established method. The dose required for the complete inactivation of HIV is not yet clear; 15 kGy or more may inactivate the virus, but this is disputed (Knaepler, Koch and Bugany 1992) and the requirement may well be higher. In bone-patellar ligament-bone grafts 30 kGy has been shown to be effective against HIV (Fideler et al 1994).

Gamma irradiation may affect the mechanical properties of bone. There have been several studies, some on animal rather than on human bone (Triantafyllou, Sotiropoulos and Triantafyllou 1975; Pelker, Friedlaender and Markham 1983; Roe, Pijanowski and Johnson 1991; Guo, Xia and Lin 1991; Itoman and Nakamura 1991; Knaepler, Haas and Puschel 1991) and some others on trabecular bone (Knaepler et al 1991; Anderson, Keyak and Skinner 1992). Beam specimens of cortical bone have often been used (Triantafyllou et al 1975; Komender 1976; Bright and Burstein 1978), but it is difficult to produce specimens with uniform mechanical characteristics (Pope and Outwater 1974; Lotz, Gerhart and Hayes 1991) since bone is anisotropic and the material properties vary with position within the bone.
The orientation of the specimen is also important even if it is taken exclusively from the diaphysis (Evans and Vincentelli 1974; Reilly and Burstein 1975). Longitudinal beam samples from diaphyseal bone differ in properties from the medial to the lateral side and from the anterior to the posterior direction (Sedlin and Hirsch 1966). This contributes to any difference in material properties between control and study groups if not accounted for in the design of the study. Komender (1976) alone mentions this potential problem. He reported “no statistically significant” difference in material properties in an unspecified number of untreated samples taken from the femoral diaphysis, but gave no data to support his conclusions.

To overcome the problems of differences in properties between samples, we have described a new type of specimen (Hamer et al 1995). A transverse section of the femoral shaft is roughly teardrop-shaped, with the linea aspera projecting posteriorly (Fig. 1). The medial and lateral halves of the cross-section are near to mirror images over several centimetres of the midfemoral shaft. The use of thin transverse sections of this part of the bone provides samples which vary little in shape, dimensions, or composition, and adjacent samples contain elements from all parts of the cross-section. Variations in properties within an individual ring should also be present in its neighbours.

We did not derive absolute values of elastic modulus and strength for the ring samples, tested in three-point bending, but used the measured values of the following parameters:

1) gradient of elastic region, or \( W/\delta \) (\( W = \text{load (N)}, \delta = \text{deflection (mm)} \));
2) load at limit of proportionality (elastic limit) or \( W_{\text{prop}}(\text{N}) \);
3) maximum load sustained or \( W_{\text{max}}(\text{N}) \); and
4) work done to point of maximum load (Nmm).

Test results with ring samples were more consistent than those with beam specimens; it seemed that the effect of external treatment would be more apparent in relatively small numbers of specimens. To study the biomechanical effects of various methods of preparation of large load-bearing allografts we used this ring model to examine the effects on cortical allograft bone of:

1) gamma irradiation at ‘standard’ doses (28 to 30 kGy);
2) freezing to -70°C and thawing to room temperature;
3) a combination of 1) and 2); and
4) increasing doses of gamma irradiation from 6.8 to 60 kGy.

**MATERIALS AND METHODS**

**Sample preparation.** Femora were obtained from seven donors at post-mortem. None had malignancy of any type and bone with gross osteoporosis was excluded. Serological screening for hepatitis B and C viruses and HIV was performed and the bones were stored at 4°C until negative results were available. They were then stripped of soft tissue in a class 1 cabinet, and the distal and proximal ends of the femora removed with a small bandsaw. The remaining femoral diaphyses were cut into 30 mm long sections which were then cut by a low-speed diamond-cutting-grinding apparatus (EXAKT; Mederex, Bath, UK) into 10 to 12 ring sections, approximately 1.6 mm thick. All the cutting was carried out with water cooling to prevent any rise of temperature which may lead to irreversible damage (Bonfield and Li 1968). The direction of the cut was always perpendicular to the long axis of the femur. All samples were kept wet, placed in heat-sealed polyethylene envelopes and stored at 4°C until required. Several sets of rings were produced from each donor femur; within each group, alternate rings were assigned to treatment and control groups.

**Irradiation.** Samples undergoing irradiation were exposed at room temperature to a 60Co source for approximately 11 hours in a commercial plant (Swann-Morton, Sheffield, UK) to give a dose of 28 to 30 kGy. Specimens were passed through the plant twice to give a 60 kGy dose, and exposed for the appropriate period for other doses. Perspex dosimeters were placed adjacent to the samples to record the actual dose.

The passage of irradiation through tissue is a two-stage process. First, energy is transferred from the photons into kinetic energy of high-speed electrons derived from atoms within the tissue, known as the ‘Compton interaction’ (Johns and Cunningham 1971); energy is then released from these electrons as they slow down in the tissue. The dose received by the tissue is related to the damage done by the Compton electrons. One such electron may ionise 15 000 additional atoms and most of the radiation effect occurs in the region of these atoms.

Compton electrons have a definite ‘range’ within tissue, and at depths of less than this range the full dose of secondary ionisation will not be received. This depth is known as
the ‘build-up’ region, which in bone is approximately 2 mm. Rings less than 2 mm thick exposed to gamma irradiation may not receive the full effect. The samples were therefore sandwiched between two 3 mm thick aluminium plates, the atomic number and density of which were very similar to bone (bone $N = 14.5$; aluminium $N = 17$; bone density = 1.7 to 2.0 $g/cm^3$; aluminium density = 2.7 $g/cm^3$). The plates provided material for dose build-up and held the bone rings in a standard position for irradiation. When large segments of bone are irradiated, the dose build-up is confined to the surfaces, and it is likely that most of the bone receives the expected dose. We could find no records of other studies on the effects of gamma irradiation on small bone samples which mentioned the problem of dose build-up.

**Freezing.** In a freezer large sections of femur cool faster on the surface than within, leading to a temperature gradient across the bone which may produce ‘thermal stresses’ and affect the mechanical properties. Ice crystals may form during freezing; this has been reported for other biological tissues, notably heart valves (Bank and Brockbank 1987). As the crystals form and expand microscopic damage may be caused: in cortical bone longitudinal cracks appear if freezing is too fast and cause a reduction in hoop strength (Bright and Burchardt 1983). To freeze the ring samples at a rate similar to that of the bone within an intact allograft segment, each ring was held between two segments of bone during the freeze-thaw cycle (Figure 2).

**Mechanical test.** We used a three-point bending jig in an Instron type 1026 mechanical testing machine (Instron Ltd, High Wycombe, UK), which converted the upward displacement of the crosshead to a bending load. To produce the most rigid jig possible, the components were machined from solid bars. Rollers were incorporated to minimise any shear forces and deformation of the samples at the point of contact. The gauge length of the jig was 24 mm.

Testing was done with a crosshead speed of 0.5 mm/min. The samples were removed from their polyethylene envelopes just before testing at a room temperature of 19 to 21°C. The maximum period of time of exposure to air and therefore at risk of drying was two minutes. The specimens were loaded and unloaded once within their linear elastic regions, and then loaded to failure. The mechanical parameters listed above were determined from the load-deflection output of the machine. Gradients of the elastic regions were measured by the ‘best-fit’ line and the area under the curve corresponding to the work done to $W_{\text{max}}$ by a digital planimeter.

**RESULTS**

**Comparison of fresh, fresh-frozen and irradiated bone** (28.5 to 30 kGy). We cut 233 rings from the femora of six donors (five male, one female; age range 16 to 83 years). They were allocated to control and treatment groups as shown in Table I.

To examine the effect of each treatment on the four mechanical parameters measured we used multifactorial analysis of variance (statgraphics for Windows v 1.0; Manugistics Inc. Rockville, Maryland) with 99% confidence limits with treatment and donor as factors. Throughout the analyses, the effect of different donors was small. The results for treatment type are shown in Figures 3 to 6. In each of these graphs, the mean values for the pooled data from all the donors are shown with ± 99% confidence intervals.

Freezing and thawing to -70°C had no effect and no treatment had any effect on the gradient of the elastic regions ($W/\delta$) of the samples. Gamma irradiation and gamma irradiation combined with freezing-thawing significantly decreased $W_{\text{prop}}$, $W_{\text{max}}$, and the work done to $W_{\text{max}}$. There was no extra effect when freezing-thawing was combined with gamma irradiation. Table II gives the percentage values of each parameter, taking control values as 100%.

**Increasing doses of gamma irradiation.** We used 132 rings cut from the femora of seven donors (six male, one female; age range 16 to 83 years). They were allocated to

![Fig. 2](https://example.com/fig2.png)

**Fig. 2**

Ring specimen held between two sections of femur for freezing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma irradiation</td>
<td>51</td>
</tr>
<tr>
<td>(28.5 to 30 kGy)</td>
<td></td>
</tr>
<tr>
<td>Freezing-thawing</td>
<td>38</td>
</tr>
<tr>
<td>Gamma irradiation + freezing-thawing</td>
<td>27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>116</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td><strong>117</strong></td>
</tr>
</tbody>
</table>
We again used multifactorial analysis of variance with treatment and donor as factors with 99% confidence limits to examine the effect of each dose of gamma irradiation on the four mechanical parameters measured, compared with control bone samples. There was no dose-related effect on the gradient of the elastic region. There was a reduction in $W_{\text{prop}}$ to 45% of the mean control value, but only at 60 kGy. $W_{\text{max}}$ and work to $W_{\text{max}}$ were related to the dose received. Both decreased.

### Table II. Mean values (percentage; control = 100%) for each mechanical parameter in the three treatment groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>$W/\delta$</th>
<th>$W_{\text{prop}}$</th>
<th>$W_{\text{max}}$</th>
<th>Work done to $W_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezing-thawing</td>
<td>NS*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Irradiated</td>
<td>NS</td>
<td>83</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>Irradiated + freezing-thawing</td>
<td>NS</td>
<td>83</td>
<td>67</td>
<td>37</td>
</tr>
</tbody>
</table>

*not significant

### Table III. The allocation of samples to irradiation dose

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma dose (kJy)</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>6</td>
</tr>
<tr>
<td>9.5</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
<tr>
<td>Control</td>
<td>66</td>
</tr>
</tbody>
</table>

Comparison of gradient of elastic region in each treatment group.

Limit of proportionality ($W_{\text{prop}}$) in each treatment group.

Maximum force sustained by rings ($W_{\text{max}}$) in each treatment group.

Work done to $W_{\text{max}}$ by each treatment group.

Fig. 3

Fig. 4

Fig. 5

Fig. 6
with increasing dose, the effect being more significant with work done to $W_{\text{max}}$ than with $W_{\text{max}}$ alone (Fig. 7).

**DISCUSSION**

We have been careful to standardise treatment and test conditions. The specimen type and method of testing have better reproducibility than ‘conventional’ beam samples tested in three-point bending. The methods of irradiation and deep freezing have been developed to simulate the processes which bone grafts undergo in normal preparation. We are confident that the changes in the behaviour of the bones after each treatment can be ascribed to treatment, and not to any other uncontrolled factors.

We agree with other authors that freezing and thawing bone to -70°C have no effect on the measured mechanical properties of cortical bone. If microcracks are produced by the rapid formation of ice crystals, they have no deleterious effect. Like others, we have found that gamma irradiation has an effect on the mechanical properties of bone. Different effects are seen in the elastic and plastic regions. At standard doses of irradiation, the elasticity of the bone is unaffected, but the capacity to absorb work and strength (equivalent to $W_{\text{prop}}$) are decreased. Although the gradient of the elastic region remains unchanged, there is a reduction in the yield point ($W_{\text{prop}}$). The loss of plastic behaviour, i.e., embrittlement, is shown in Figure 8.

We also demonstrated a dose-dependent reduction in the strength of the samples (reflected by a fall in $W_{\text{max}}$). Even at 60 kGy, however, the elastic modulus of the bone was unaffected.

The strength of bone depends on the mineral fraction, the collagen fraction and on the interaction of the two. There is evidence that the degree of mineralisation of bone affects elasticity while its plastic behaviour depends on the collagen content (Burstein et al 1975). Our findings therefore suggest that irradiation affected the collagen in our samples. Similar doses of irradiation have been shown to affect the collagen in skin or tendon by the breakdown of molecules into smaller subunits or by disorganization of the secondary structure of the triple helix (Bowes and Moss 1962; Bailey 1968). It has been suggested that irradiation may also affect collagen intermolecular crosslinks and therefore its mechanical stability (Bright and Burstein 1978). We are investigating the effect of gamma irradiation on mature bone crosslinks (pyridinoline and deoxypyridinoline), as it has been shown in vitro that they are susceptible to degradation by electromagnetic radiation (Fujimori 1985).

In clinical practice, as long as the allografts are subjected to ‘normal’ loadings and loading rates, so that the loads are within the elastic region of their behaviour, then graft collapse or fracture will probably not occur. High bending loads applied to cortical struts, for example, should be avoided, as this mode of loading may exceed the elastic limit of the graft in bending. The biological behaviour of such large allografts is poorly understood, and it is not yet clear how irradiation affects graft incorporation.

We would like to thank Mr Keith Bumford for his helpful advice concerning irradiation. We also thank Professor John Currey of York University and Dr Clive Lee of Exeter University for their helpful comments. We are also grateful to the Wishbone Trust and the research committee at the Northern General Hospital for financial support.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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