Study of Human Cortical Bone and Demineralized Human Cortical Bone Viscoelasticity

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ABSTRACT: The knowledge of human bone viscoelasticity is an important issue for defining semirigid calcified tissues implants. A very sensitive technique was used to investigate bone viscoelasticity: the thermally stimulated creep method. A study of demineralized human bone was performed to determine the molecular origin of bone viscoelasticity. The thermally stimulated creep spectra of bone and demineralized bone, at the hydrated state, present a similar shape with one main retardation mode located at −133 and −120°C, respectively. This mode is shifted toward higher temperatures after dehydration, revealing the existence of another mode at around −155°C. The analysis of elementary spectra of bone and demineralized bone has shown that retardation times follow an Arrhenius equation, and that two compensation phenomena are observed with comparable compensation parameters. The first compensation phenomenon, which corresponds to the main retardation mode, was attributed to motions of water molecules located inside the collagen triple helix. The second compensation phenomenon, which reveals the existence of another relaxation mode at higher temperatures, was assigned to movements of hydrophilic side chains bound to water molecules. As for the mode observed at around −155°C, it was associated with motions of aliphatic side chains. Overall, bone viscoelasticity originates from the organic matrix.
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Key words: human cortical bone; human cortical bone collagen; viscoelasticity; retardation spectra; thermally stimulated creep

INTRODUCTION

In the application of a synthetic material as artificial bone, it is required that the implant be biocompatible, resistant to corrosion, and have adequate mechanical properties (fracture toughness and fatigue strength). For these reasons, alloys such as austenic stainless steel, Co-Cr alloy, Ti-Al alloy, etc., have been widely used. However, for all these "hard" implant materials, one intrinsic problem is implant loosening: with time, the implant material tends to become detached from the bone. This loosening is attributed to the implant material Young's modulus, which is generally much larger than the one of bone. One approach to overcome this problem would be to use an implant material with an elastic modulus similar to the one of bone.

Following this guideline, "soft" composite materials intended for use as implants have been synthesized and tested. Most of these materials are composed of resin impregnated with ceramic particles. By changing the ceramic particle content, materials with elastic moduli similar to that of bone have been obtained.[1,2] But there is a possibility that the viscoelastic characteristics of the

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soft implant material may be much different than those of bone, which would raise a new problem for a soft implant material.

Presently, the relaxational behavior of bone is not well known. Even if viscoelasticity has long been recognized as one of the important properties of bone,[3–6] most works are related to the mechanical strength and modulus of elasticity of bone.[7] Bone viscoelasticity cannot be measured easily, because the strain is dominated by elasticity. A new technique has been used to investigate it: the thermally stimulated creep (TSCr) method. The purpose of the present investigation is to determine the viscoelastic response of bone and, in the future, to compare it with the one of soft implant materials.

In TSCr, samples are subjected to a static mechanical torsion. Bone studies by torsion have been reported by several authors. In 1967, Bonfield and Li[8] examined the torsional properties of filaments (254-μm diameter) of bovine tibial cortical bone and found a nonelastic effect for stresses greater than 3.9 $10^6 N/m^2$ and a shear modulus of 5.6–6.1 $10^9 N/m^2$. In 1975, Reilly and Burstein[10] studied the elastic and ultimate properties of human and bovine cortical bone and found a shear modulus for human femoral compact bone of 3.28 $10^9 N/m^2$. In the same period, Frasca and Katz[9] and Lakes and Katz[6] examined the dynamic torsional response of human single osteons and human cortical bone. Finally, Ascenzi et al.,[11] who were able to isolate single osteons since 1967, studied the torsional properties of single selected osteons in 1994.

The main role of cortical bone is to ensure a structural framework, but it has other functions such as protecting vital organs (the central nervous system), to regulate calcium exchanges, and to contain the marrow where blood cells are formed. Cortical bone may be divided into a material part and a living part: the bone matrix and the bone cells. The bone cells draw up or resorb the bone and the bone matrix is the support tissue. The bone matrix can be considered as a multicomponent biological composite material composed of mineral (amorphous and crystalline apatite), organic matrix (collagen and protein molecules), and water phase.[12–14] Cortical bone can also be considered as a hierarchical solid composed of structural elements which in themselves have a discrete structure. This complex organization makes cortical bone an anisotropic material. The organic phase primarily consists of type I collagen fibers which possess many of the characteristics of polymeric materials.[15] The mineral phase is composed essentially of calcium hydroxyapatite crystals [$Ca_{10}(PO_4)_6OH_2$] that precipitate around the collagen fibers[16,17] and exhibit a behavior similar to that of ceramic materials.[13] Water (bound and unbound) facilitates the interactions between the aforementioned phases.[18,19]

Numerous studies showed that the relaxation phenomena in bone are changed by water content.[4,6,20] The fact that collagen mechanical properties are greatly affected by water content[21,22] and that collagen exhibits viscoelastic behavior is suggestive of a possible role of collagen molecular motions in determining bone's viscoelastic response. We verified this possibility by comparing bone TSCr response with the one of bone organic phase. This study allowed us, on one hand, to obtain the relaxational behavior of bone, and on the other hand, to determine the origin of bone viscoelastic response.

EXPERIMENTAL

Material

Bone samples used in this work were obtained from the mid-diaphysis of a 20-year-old human femur. Samples were taken by cutting bone with a diamond circular saw. This cutting was performed under tap water to avoid the denaturation of matrix proteins by frictionally generated heat. The resultant pieces were shaped into rectangular plates sized $7 \times 70 \times 0.6$ mm^3 on average. The longest edges were parallel to the longitudinal axis of the bone (Fig. 1).

To get a bone organic matrix specimen, bone samples were demineralized. They were immersed in 0.5*M* ethylenediaminetetraacetic acid, pH 7.4 at 4°C for 9 days. The ethylenediaminetetraacetic acid solution was replaced daily to avoid protease action. By using atomic absorption spectroscopy, we have verified that after 9 days, bone samples were totally demineralized. Our experimental procedure was comparable to that of Bowman et al.[23]

Before measurements, the specimens were dried for several days in a helium atmosphere at room temperature, inside the measurement cell. Afterward, the water content was 0.07 g water/g bone, for bone, and 0.11 g water/g organic matrix, for bone organic matrix.

Complex TSCr Spectra

The set-up for TSCr has been described in several publications.[24–26] The torque stress is applied

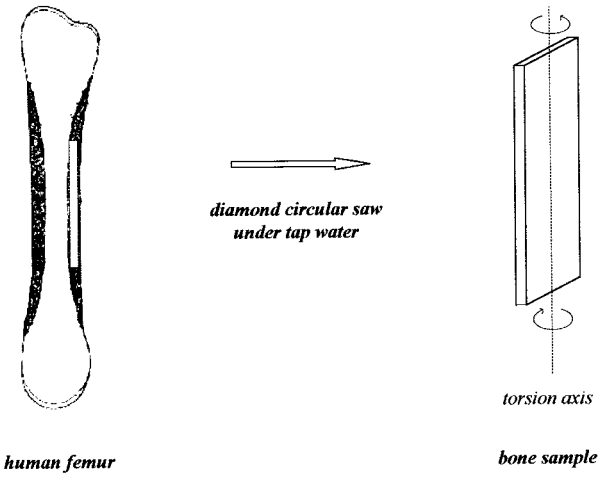

Figure 1 Samples were prepared by cutting human bone femur with a circular saw under a circulation of water. Samples thus obtained were tested by shear creep, their long axis corresponding to bone long axis.

through a spring to the sample. Two different springs were used to match with the strength of bone and demineralized bone, with constant stiffness k_S of 5.7 10^{-2} Nm/rad and 2.86 10^{-3} Nm/rad, respectively.

The TSCr principle is the following: a torque stress is applied to a sample at a given temperature during 2 min. Then the sample is quenched down to a lower temperature to freeze the orientation of molecular segments, and the stress is removed. [During 2 min, the elastic deformation recovery may be observed: none!] Finally, a linear increase of the temperature is applied, at a scanning rate of 7°C/min, and the anelastic deformation recovery is recorded. Thus, a complex TSCr spectrum is obtained. This spectrum is generally complex because it often results from a distribution of retardation times. The use of the fractional loading method allows one to determine the distribution of retardation times.

Elementary TSCr Spectra

The fractional loading method consists of applying the stress in a narrow temperature range, selecting a narrow distribution of retardation times. A series of elementary spectra was obtained by shifting the stress temperature along the temperature axis. In present experiments, the stress was applied at Tp for 2 min. It allowed us to orient the segments, getting retardation times lower than 2 min. Then the temperature was lowered until Td = Tp−5°C and the stress suppressed during 2 min so that segments with short retardation times could relax. Then the sample was quenched down to Tp−40°C. The anelastic deformation recovery and the anelastic deformation rate were recorded by using the same protocol as for the complex spectrum.

Analysis of Elementary Spectra

Each elementary peak can be analyzed with the hypothesis of a unique retardation time. The temperature dependence of retardation times is given by the relation:

$$\tau_i(T) = \frac{\gamma_i(T)}{\dot{\gamma}_i(T)} \tag{1}$$

where $\tau_i(T)$ is the retardation time at the temperature T for the elementary process i, γ_i (T) is the anelastic deformation recovery and $_i$ (T) is the rate of anelastic deformation recovery. Retardation times usually obey an Arrhenius equation:

$$\tau_i(T) = \tau_{oi}\exp\frac{E_{ai}}{kT} \tag{2}$$

where τ_{oi} is the pre-exponential factor and E_{ai} is the activation energy.

As dicussed below, in the Arrhenius diagram, some elementary processes converge in a common point of coordinates (T_c, τ_c). This phenomenon is called a compensation phenomenon and shows a common origin of processes that would relax at the temperature T_c with the same retardation time τ_c. From the Hoffman et al. model,[27] a compensation phenomenon is due to cooperative molecular movements: chain segments with increasing length relax with increasing activation energies and entropies.

RESULTS

Complex TSCr Spectra

As stated previously, relaxation phenomena in bone and in demineralized bone are dependent on water content. The relaxation spectra of bone were obtained at three different hydration levels, which were obtained by conserving the sample 4 days in helium (0.073 g water/g bone), 12 h under vacuum (0.047 g water/g bone), and 10 min at 110°C (0.01 g water/g bone). The corresponding spectra, presented in Figure 2, were obtained by

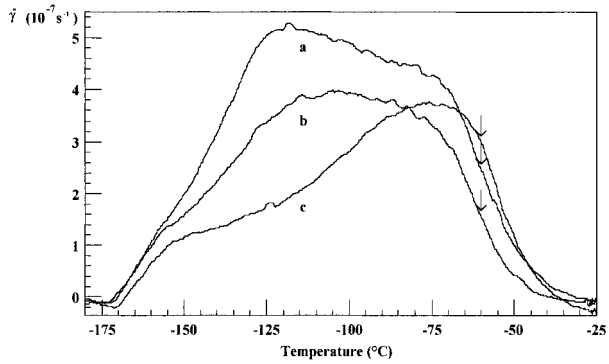

Figure 2 TSCr complex spectra of human bone at three different hydration levels: (a) 0.073 g water/g bone, (b) 0.047 g water/g bone, and (c) 0.01g water/g bone (the arrows symbolize the stress temperature).

applying 20° to the torsion pendulum at −60°C. At the hydrated state, one main loss peak is observed at −120°C. As hydration decreases, this peak is shifted toward higher temperatures and a low intensity peak appears at around −155°C. This weak peak is independent of the hydration level. At the dehydrated state, the main loss peak appears at around −75°C.

Demineralized bone was studied at two different hydration levels and the complex spectra were obtained by applying 42° to the torsion pendulum at −60°C (Fig. 3). The hydrated state was obtained by conserving the sample for 2 days in helium (0.11 g water/g organic matrix) and the dehydrated state by maintaining the sample for 1 min at 180°C (0.01 g water/g organic matrix). At the hydrated state, one main loss peak is observed at −130°C. As for bone, this main loss peak is shifted toward higher temperatures and a

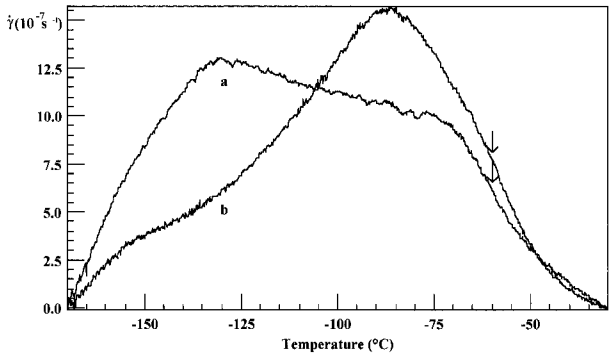

Figure 3 TSCr complex spectra of demineralized bone at two different hydration levels: (a) 0.11 g water/g organic matrix, and (b) 0.01 g water/g organic matrix.

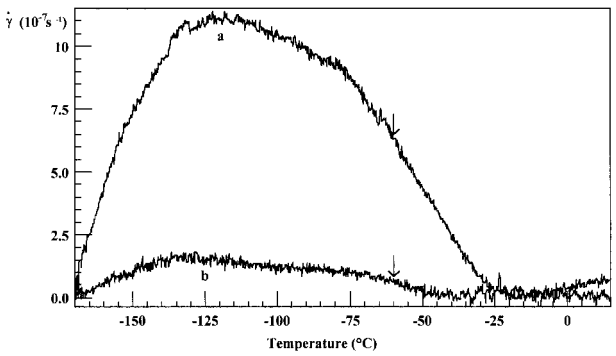

Figure 4 TSCr complex spectra of organic matrix (a) and bone (b) in the hydrated state.

low intensity peak appears at around −155°C with dehydration.

To compare loss peak intensities, bone and demineralized bone were studied using the same torsion spring (k_S = 2.86 10^{-3} Nm/rad), and the same torque angle (42°), at the hydrated state. The obtained results, which are presented in Figure 4, show that the main loss peak of bone is lower than the one of organic matrix by a factor of 7.

The lower strength of the loss peak in bone may be explained in the following way. First, the bone shear modulus is higher than the organic matrix shear modulus. The values of the modulus were estimated, at −60°C, by measuring the sample strain when the stress was applied. Shear moduli of 13.8 MPa for bone and 2.2 MPa for the organic matrix were obtained. Second, bone is more elastic than viscous; when the stress was removed at −170°C, bone elastic deformation recovery represented 75% of the initial deformation whereas organic matrix elastic deformation recovery only represented 12%.

These differences in the relaxational behavior of bone and demineralized bone result from the presence of mineral. The mineral phase increases bone shear modulus and decreases bone viscoelastic response magnitude. As for bone viscoelastic response, the comparison of the complex TSCr spectra obtained for bone and demineralized bone shows that bone and organic matrix loss peak temperature positions are similar and that their evolution with hydration is comparable. Consequently, the viscoelastic response of bone actually seems to come from the organic matrix, which is mainly collagenic. A more detailed study of this viscoelastic response is proposed using the fractional loading method.

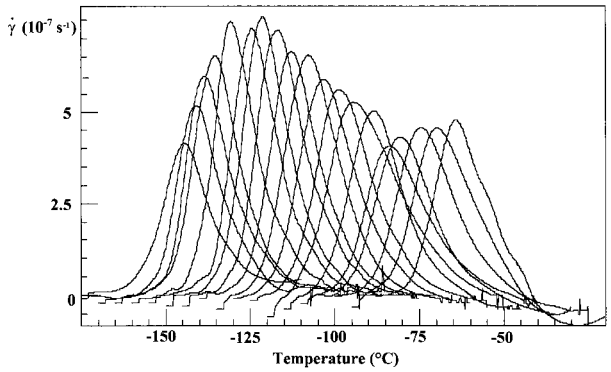

Figure 5 TSCr elementary spectra of human bone in the hydrated state.

Elementary TSCr Spectra

For this study, bone and organic matrix were analyzed at the hydrated state. The series of fractional loading spectra were obtained by shifting the polarization window along the temperature axis from −150 to −60°C, in steps of 5°C (cf. Fig. 5), for bone, and from −150 to −60°C, in steps of 10°C (cf. Fig. 6), for the organic matrix.

In both cases, the envelop of the elementary spectra confirms the existence of one main loss peak at around −120°C. The analysis of each elementary peak and the semi-logarithmic representation of τ_i versus T^{-1} show that, in both cases, retardation times obey an Arrhenius equation and that two compensation phenomena are observed (cf. Figs. 7 and 8).

For bone, the compensation parameters are: T_c = 5°C and τ_c = 3 10^{-6} s, for the elementary spectra obtained from −135 to −110°C and numbered 4 to 9; and T_c = 63°C and τ_c = 2 10^{-6} s for the elementary spectra isolated between −90 and −60°C, and numbered 13 to 19.

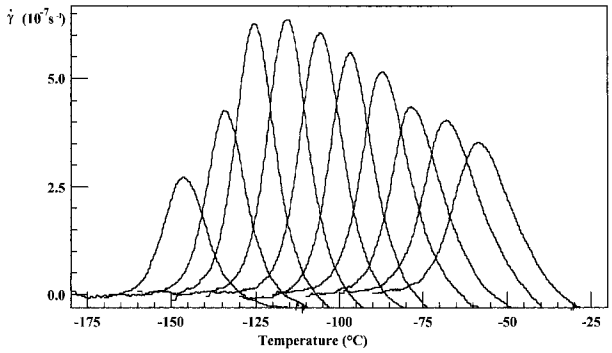

Figure 6 TSCr elementary spectra of demineralized human bone in the hydrated state.

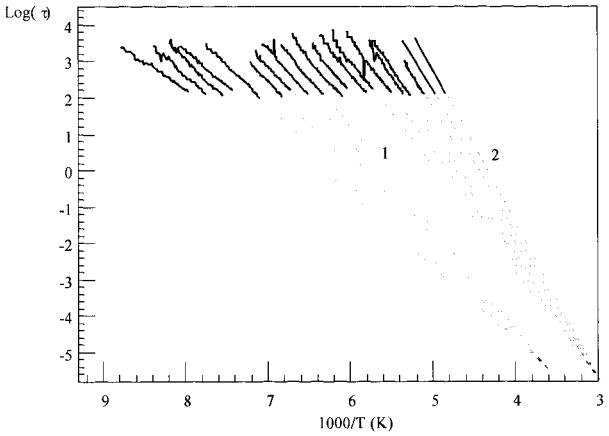

Figure 7 Distribution of retardation times obtained by the analysis of elementary spectra in bone.

For the organic matrix, the compensation parameters are: T_c = 10°C and τ_c = 6 10^{-6} s, for the spectra obtained from −140 to −120°C and numbered 2 to 4; and T_c = 90°C, and τ_c = 10^{-7} s for the spectra isolated between −110 and −90°C, and numbered 5 to 7.

DISCUSSION

As mentioned previously, demineralized bone is mainly collagenic. Collagen is a macromolecule consisting of polypeptides organized in the form of a triple helix. The important water absorption of collagen is due both to its triple helix structure and to its chemical composition (large concentration of hydrophilic groups: C=O, N—H, COOH, OH, etc.). Nomura et al.[21] have identified four

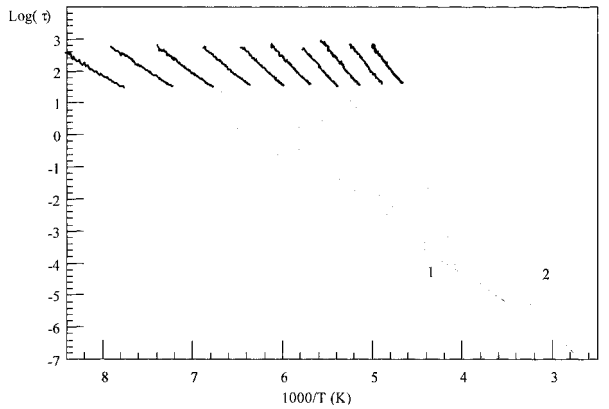

Figure 8 Distribution of retardation times obtained by the analysis of elementary spectra in demineralized bone.

regimes in the hydration of human dura matter collagenous tissues: the first one (0–0.07 g/g) is associated with structural water located in the triple helix, the second one (0.07–0.25 g/g) with water bound to the polar side chains and located in the interhelical regions, the third one (0.25–0.45 g/g) is a transition region in which both bound and free water are sorbed, and the last one (>0.45 g/g) is associated with free water (freezable water). Using dynamic mechanical spectroscopy, they observed two very weak relaxations (at 0.01 g water/g collagen and at 1 Hz) located at −130°C (labeled γ) and at −10°C (labeled β_2).

These two relaxation processes may be compared with the modes observed at −155 and −85°C by TSCr on demineralized bone, at a comparable water content (cf. Fig. 3). Moreover, as for Nomura et al.,[21] the β_2 mode shifts to lower temperatures when the water content is increased. Consequently, the mode observed at −130°C for demineralized bone at 0.11 g/g water can be associated with the β_2 mode described by Nomura et al.[21] By analogy with low temperature broad peaks, frequently observed in hydrated polymers[28] and associated with water molecule movement, the β_2 mode may be associated with the relaxation of water molecules localized inside the collagen triple helix. Moreover, the analysis of the fine structure of demineralized bone in the temperature range of the β_2 mode shows that the corresponding molecular movements are cooperative.

In bone, a similar compensation phenomenon is observed. This compensation phenomenon, isolated in the same temperature range as the main loss peak observed at −120°C, confirms that this mode has the same origin as the one observed in demineralized bone.

As for the γ mode, we could not use the fractional loading method to decompose the loss peak observed at −155°C, because this mode is too low in temperature. Nevertheless, Lamure et al.[29] managed to obtain the γ mode fine structure on dried cartilage collagen. They observed one compensation phenomenon in the temperature range of the γ mode. The corresponding compensation temperature, close to collagen glass transition temperature, allowed them to ascribe this mode to molecular motions that are precursors of the glass transition. Because this mode is not influenced by hydration, it was attributed to motions of neutral side chains, like aliphatic side chains, as proposed by Nomura et al.[21]

In demineralized bone, a second compensation phenomenon is observed between −110 and −90°C. Even if, in this temperature range, no loss peak appears, the compensation phenomenon associated with cooperative movements reveals an additional relaxation mode. Indeed, Nomura et al.[21] have shown that a second peak, labeled β_1, sensitive to water, appears at water content above 0.1 g/g. This peak shifts to lower temperatures and becomes more intense as the water content is increased, reaching a maximum intensity at about 0.45 g/g. So, they have attributed this mode to motions of water molecules bound to the polar side chains and located in the interhelical regions. In our study, the fine structure of this mode gives a compensation temperature of 90°C. This temperature is close to the collagen glass transition temperature.[30] Consequently, the movements corresponding to this mode are precursors of collagen glass transition. Moreover, as this mode is influenced by hydration, it corresponds to motions of side chain hydrophilic groups. In bone, a comparable compensation phenomenon appears between −90 and −60°C with a lower compensation temperature (63°C). The differences observed for bone and demineralized bone may come from the presence of the mineral located between the triple helix and between the head and tail of two aligned triple helices, as if mineral acted like a plasticizer on collagen, decreasing its glass transition.

CONCLUSION

The low temperature viscoelastic response of bone was detected using TSCr. The comparison of bone and demineralized bone responses allowed us to confirm that bone elasticity originates in the mineral phase and bone viscosity comes from the organic phase and depends on hydration level.

At the hydrated state, the complex TSCr spectra display one main retardation mode, labeled β_2, and located at −133 and −120°C in bone and demineralized bone, respectively. The shape of this mode is similar for the two materials. After dehydration, this mode is shifted toward higher temperature, and another retardation process, labeled γ is found at around −155°C.

The very low temperature mode was attributed to motions of neutral side chains. As for the main retardation mode, it was assigned to motions of water molecules located inside the collagen triple helix.

The analysis of elementary spectra, obtained by fractional loading on bone and demineralized bone, has revealed the existence of two compensation phenomena for each sample. The first compensation phenomenon appears in the same temperature range as the β_2 mode, whereas no loss peak corresponds to the second compensation phenomenon. This second compensation phenomenon is probably related to another relaxation mode, β_1, reported in the literature. This higher temperature mode was associated with movements of hydrophilic side chains bound to water molecules.

This study has allowed us to quantify mechanical properties of bone with, in particular, a high elastic modulus which allows bone to resist high stresses, and a viscous component which is responsible for the dissipative effect under impact. The intent of this work was to propose a model of the viscoelastic properties of bone at a given frequency and in a temperature range that permits maintenance of a hydration level as close as possible to physiological conditions. In the future, the distribution of retardation times might be used to simulate the behavior of bone at the physiological temperature to determine the origin of dissipation effects.

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