CHANGES IN THE PROTON RELAXATION TIME AND WATER CONTENT OF BONE TISSUE DURING CONSERVATION BY COLD

D. G. Takhavieva, V. I. Rokityanskii,* and Yu. V. Yakimov

UDC 615.361.71.014.413

The spin-lattice (T_1) and spin-spin(T_2) relaxation times and the bound water content were investigated by the nuclear magnetic resonance (spin echo) method in human cadaveric cortical bone tissue preserved for various periods at −25°C. The total water content was determined. The parameters investigated undergo changes during bone conservation. During storage for up to 4 months the value of T_1 corresponds to the values for fresh bone, with an increase in the content of total water and bound water. During more prolonged storage the changes are qualitatively different in character and by the twelfth month, in conjunction with some drying of the bone, the value of T_2 is increased. The content of bound water is reduced. These results suggest substantial disturbances of the water balance of bone tissue and partial destruction of the complexes of bound intracellular water.

In investigations of the viability of conserved bone [1-6, 8] the investigation of the state of the intracellular water has been neglected.

The object of this investigation was to study the dynamics of the proton relaxation times and the water content in conserved human bone by means of the spin-echo method, one of the nuclear magnetic resonance (NMR) techniques.

EXPERIMENTAL METHOD

Altogether 303 specimens of human cadaveric cortical bone tissue, preserved by freezing at −25°C for different periods (1-2, 3-4, 6, and 12 months) were studied. Fresh bone from the same segment (the tibial diaphysis) was used as the control. The bone was ground in a porcelain mortar to a particle size of about 1 mm and packed firmly into special glass tubes (samples weighing 3-4 g) which, after weighing, were placed in the measuring cell of an NMR apparatus. The spin echo signals were recorded by Hahn's method [7]. The values of the spin-lattice (T_1) and spin-spin (T_2) relaxation times were determined.†

The contents of the tubes were dried to constant weight and the total water content in the specimen determined. The quantity of bound water was calculated by the equation of Solomon and Blombergen.

EXPERIMENTAL RESULTS

Fresh human cortical bone from a middle-aged individual contains 152 ± 5 mg water/g dry substance. The spin-lattice relaxation time (T_1) was 103 ± 2 msec and the spin-spin relaxation time (T_2) 28 ± 0.5 msec. The content of bound intracellular and intrafibrous water in the bone was 15 mg/g dry substance.

* Deceased.

† These measurements were carried out in the Department of Physics, Kazan' Pedagogic Institute.

Kazan' Scientific-Research Institute of Traumatology and Orthopedics. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 77, No. 2, pp. 92-94, February, 1974. Original article submitted May 30, 1973.

Bone preserved for up to 4 months showed an increase in the total water content. At the same time the value of T_1 corresponded to that for fresh bone, as a result of an increase in the content of bound water to 16 mg/g dry substance. These indices are evidence that the bone preserves its biological properties for this time.

The increase in the total water and bound water content, associated with a stable value of T_1, indicates the preservation of that part of the water which is hydrated around the bone ions and biomacromolecules, as a result of the preceding freezing.

At later periods of conservation the changes observed in the bone were qualitatively different. A sharp decrease in the total water content, compared both with the previous periods of conservation and with the corresponding parameters of fresh bone, was found. The decrease in the total water content during conservation of the bone for 6 months was accompanied by a linear shortening in the value of T_1 to 90 ± 3 msec, following the change in the water content, while T_2 remained at the characteristic level for fresh bone. The bound water content was considerably reduced to 12 mg/g dry substance. The closeness of T_2 to the corresponding parameter for fresh bone, in conjunction with the reduced total water and bound water content, indicates the onset of changes in the biological properties of the conserved bone. Meanwhile, the more marked shortening of T_1 than at the previous times, corresponding to the decrease in the total and bound water content, together with the ratio between the bound and total water content, indicate that the changes developing in the bone at this period of conservation are still not regardable as severe disturbances.

The total water content in bone conserved for 12 months also was lower than initially, namely 123 ± 4 mg/g dry substance. The spin-spin relaxation time T_2 was increased to 36 ± 0.7 msec. The increase in duration of T_2 in conjunction with some degree of drying of the material was due, presumably, to the marked decrease in the content of bound water to 10 mg/g dry substance. Despite the appreciable decrease in the total water content, the content of bound water expressed as a relative percentage was smaller than in fresh bone. This fact is evidence of substantial changes in the mobility of some of the water, in the water balance of the bone tissue, and in the bound water both within the cells and in the fibrous structures and reflects a disturbance of the biological properties of bone conserved for 12 months. Freezing to $-25°C$ does not completely suppress metabolism in the conserved bone. The unceasing thermal movement gradually destroys the ice structures and with the course of time a deficiency of the ice structure develops. The connection between water and the biomolecules becomes less close and the holding of water by the biocolloids less strong. The balance between hydration and dehydration shifts toward dehydration of the biomolecules.

These investigations of conserved bone at the molecular level by means of the nuclear magnetic resonance (spin echo) method, now undertaken for the first time, give a deeper insight than other methods into the biological state of the bone tissue. The method used gives information on the behavior of proton spins in relation to surrounding molecules and on the bound water which is an inseparable part of the system of biomacromolecules and ions in the tissue fluids.

LITERATURE CITED

1. T. P. Vinogradova, in: Proceedings of a Seminar on Methods of Conservation and Transplantation of Homologous and Heterologous Tissues [in Russian], Moscow (1961), p. 15.
2. T. Ya. Balaba, L. N. Furtseva, and I. Ya. Zhukovskii, in: Proceedings of a Scientific Session on Traumatology and Orthopedics [in Russian], Riga (1966), p. 107.
3. N. A. Zvonkov and G. N. Zvonkova, Ortoped. Travmatol., No. 2, 29 (1968).
4. P. P. Kovalenko, Abstracts of Proceedings of the Second All-Union Conference on Tissue Incompatibility and the Conservation and Transplantation of Tissues and Organs [in Russian], Odessa (1961), p. 100.
5. D. A. Kozlova, in: Proceedings of a Scientific Session of Riga Research Institute of Traumatology and Orthopedics [in Russian], Riga (1965), p. 112.
6. V. S. Svigun, Ortoped. Travmatol., No. 12, 49 (1964).
7. E. L. Hahn, Physics To-Day, 6, 4 (1953).
8. M. Janecek, V. Horn, J. Brichta, et al., Acta Chir. Orthop. Traumat. Czechosl., 32, 422 (1965).