crystals is important in calculating the microstresses in single and polycubic crystals, both in the elastic and inelastic ranges.

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*A STUDY OF WATER IN BIOLOGICAL SYSTEMS BY O^{17} MAGNETIC RESONANCE SPECTROSCOPY, I. PRELIMINARY STUDIES AND XENON HYDRATES**

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There is no need to emphasize the importance of the physical structure of the water-substance in biological systems. Several recent papers have appeared which develop theoretical models for the structure of liquid water, and which attempt to apply these to biological systems.[1–3] These papers also review the older literature.

There have been a great number of experimental papers dealing with physical parameters of water such as viscosity, compressibility, refractivity, etc. The results of these measurements up to 1938, a period which includes the great majority of the work, have been compiled.[4]

The purpose of this paper is to present some preliminary results of a method, previously unused, for the investigation of the structure of water, and which is particularly suited to the study of water in biological systems. The method utilizes wide-line nuclear magnetic resonance spectroscopy of water enriched with the stable isotope O^{17} (spin = 5/2, quadrupole moment, $Q = -0.0265$ barns[5a–c]). In addition to water alone, the structure of liquid water containing dissolved xenon is discussed. This problem has been treated recently[6, 7] in connection with the anesthetic properties of xenon, and the general problem of anesthesia.

This method has not been exploited so far, since the natural concentration of H_2O^{17} in pure H_2O is about 0.2 M, and it is difficult to extract useful signals from this source with existing instruments. In the past few years, however, commercial sources have made water available with up to 15 per cent O^{17} and this concentration is much more amenable to chemical and physical studies on water, and has entered the range where O^{17} may be used as a tracer in biological work. At this enrichment much faster passage methods may be used in obtaining the magnetic resonance signal, and thus transient phenomena may be investigated, and the problem of signal saturation is eased. The use of the O^{17} resonance as a probe in the study of the structure of water and aqueous solutions does not require spin-echo equipment. This is because the relaxation times for O^{17} are of the order of milliseconds. Hence, direct line width measurements are sufficient to give accurate relaxation time information. Because of the low nuclear magnetogyric ratio of O^{17}, the influence of dissolved oxygen and paramagnetic ions on relaxation times per molecule or ion is 60 times smaller than for protons. This is of importance in biological work.

It will be shown in the following paragraphs that the governing factor in the relaxation times of O^{17} nuclei, the coupling of the nuclear electric quadrupole moment to its electronic surroundings, yields important and unique information about the structure of water.

Experimental.—The experiments reported in this paper were performed at a frequency of 8.14033 MHz (H_0 = 14,100 gauss) on a Varian Associates, Inc., DA-60 NMR spectrometer, using the wide-line portion of the instrument and a V-4210A variable-frequency RF unit. The sweep modulation amplitude, h_m, was kept constant at 26 milligauss throughout the measurements reported here. Saturation was carefully avoided. The effect of sweep modulation amplitude on the results is discussed below. A scan rate of 7 milligauss/sec was used with a signal response time of 0.1 sec.

Two ml of H_2O^{17} water were obtained from the Yeda Research and Development Co., Ltd., Rehovoth, Israel.[8] The O^{17} concentration was 9.9%, and the water had been normalized to the natural H^1/H^2 ratio. Xenon used in the experiments was Mathieson analytical reagent grade xenon. The sample tubes were precision bore, thick-wall, Pyrex tubes 15 mm OD × 9 mm ID.[9]

One of the sample tubes was made into a capsule of calibrated volume, 9.93 ml, into which was placed 0.0555 moles (1 ml) of the H_2O^{17}. This was degassed *in vacuo* by thaw-freezing once, and then 0.0107 moles of xenon were added to the capsule, after which it was sealed off. The amount was calculated to provide for conversion of all H_2O to solid xenon hydrate at minimum coordination at room temperature.[10] Upon agitation of this capsule at room temperature, the sample converted completely to a waxy solid. Upon warming to 27°C, the material in the capsule became a clear solution. There is no critical point for this hydrate.[11] When allowed to stand unagitated at room temperature, only a thin crust of solid hydrate formed on the surface of the liquid. In the NMR experiments, the mechanical arrangement was such that only a signal from the middle portion of the sample was observed.

The remaining 1 ml of H_2O^{17} was placed in a sample tube with an identical bore. Dry nitrogen was bubbled through it for a few minutes and a copper-constantan thermocouple was inserted with its junction below the surface of the liquid by 0.3 cm. At the time of the experiments, the pH of the water was 8.1 as measured with a Fisher pH meter using microelectrodes.

The probe equilibrium temperature was 26.7°C. Experiments were done at several temperatures, as measured with the copper-constantan thermocouple. These were not equilibria measurements, but were taken as the tube warmed or cooled toward probe temperature following external heating or cooling.

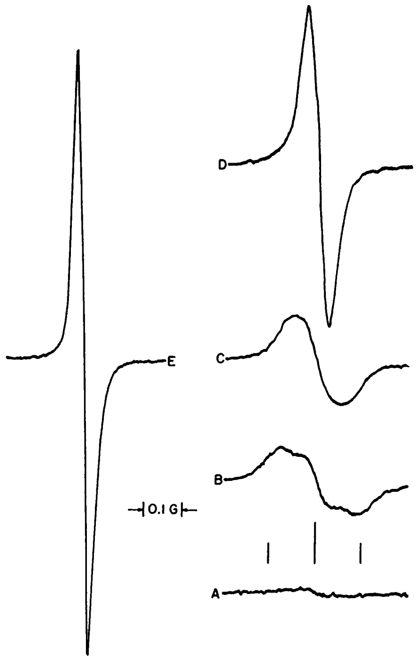

FIG. 1.—O^{17} spectra of 10% H_2O^{17} at various temperatures. These are the derivatives of the absorption spectra. (*A*) Solid ice at 0°C. (*B*) Water at 3°C. Theoretical spin-spin *absorption* triplet with $J = 73.5$ *Hz* is indicated underneath. (*C*) Water at 13°C; (*D*) water at 27.6°C; (*E*) water at 65°C.

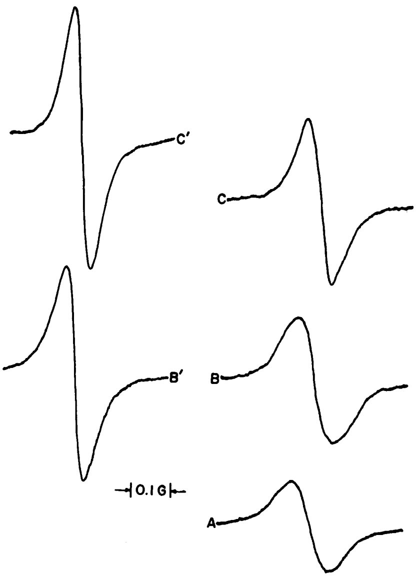

FIG. 2.—O^{17} spectra of xenon-O^{17}-hydrates. These are the derivatives of the absorption spectra. Gain is slightly different than Fig. 1. (*A*) Solid hydrate at 21°C. (*B*) Water at 21°C. (*B'*) Xenon dissolved in water at 21°C. (*C*) Water at 27.6°C. (*C'*) Xenon dissolved in water at 27.6°C. (*C* and *C'*) Spectra taken after samples were at this temperature for 50 min.

Results.—Representative spectra for pure water at various temperatures are shown in Figure 1. The results of these measurements can be summarized briefly. Ice below 0°C gives no signal. This is in accord with results presented by Rabideau and Jackson.[12] At 0°C, however, it was found that apparently solid ice gave a very small signal with an anomalously small $(\Delta H)_{m.s.}$ (width between maximum points on the derivative curve which is equivalent to the width between points of maximum slope on the corresponding absorption curve), compared with higher temperature spectra. This spectrum may be due to some liquid water trapped in the ice, but the line width is still unusually small. Below 4°C the signal is an unresolved complex as shown in Figure 1. Above 4°C to the highest temperature attained, 75°C, the line narrows considerably and changes shape. The line shape above 4°C is between Gaussian and Lorentzian as judged by the ratio of maximum positive slope to maximum negative slope[13] and a second moment analysis.[14] If γ is the magnetogyric ratio, the following relations may be derived relating the transverse relaxation time, T_2, to $(\Delta H)_{m.s.}$:

$$\text{Gaussian:}\quad 1/T_2 = 1/\sqrt{2}\pi(\Delta H)_{m.s.}$$

$$\text{Lorentzian:}\quad 1/T_2 = \sqrt{3}/2\gamma(\Delta H)_{m.s.}$$

Preliminary results obtained by graphing log $(\Delta H)_{m.s.}$ versus $1/T$, where T is the absolute temperature, in order to obtain an activation energy for $1/T_2$ reveal a plot which is a straight line from 4 to 30°C. At 30°C there is a distinct break in the line. An analysis of these experiments will be published in a later paper.

The sweep modulation amplitude, h_m, affects the shape of the detected signal. Curves relating this artificial broadening of the derivative curve to the ratio $(\Delta H)_{m.s.}/h_m$ have been published.[15] These indicate that corrections are negligible in the experiments reported here. The variation of the steady magnetic field across the width of the sample (the contribution of the field inhomogeneity to the line width and thus to $1/T_2$), was measured and this correction was also found to be negligible.

Assuming the H_2O^{17} line to be between Gaussian and Lorentzian, it was found that $1/T_2$ at 25°C was 240 sec^{-1}. The reciprocal of the spin-lattice relaxation time, $1/T_1$, may be derived from this by correcting for proton exchange broadening[16] which makes $1/T_2$ differ from $1/T_1$ at pH $= 8.1$. This results in

$$[1/T_2]_{\text{corr.}} = 1/T_1 = 195\ \text{sec}^{-1},$$

where $[1/T_2]_{\text{corr.}}$ is the value of $1/T_2$ obtained from line width measurement and corrected for exchange using Figure 6 of reference 16.

An observation that was made repeatedly during the course of this investigation was that when water, initially at a higher or lower temperature, was allowed to reach probe temperature, this temperature was arrived at quite rapidly, but upon reaching it the spectra continued to change shape slightly as a function of time. This change manifested itself in a sharpening of the lines at constant temperature. The effect is discussed below.

The spectra of the xenon hydrate sample are shown in Figure 2. The solid hydrate displays a somewhat broadened waterlike peak which has no evident chemical shift from pure water (Fig. 2*A*). Allowing this material to go over into a solution of xenon in water by raising the temperature a few degrees resulted in a sharpening of the line. However, it remained broader than the H_2O line at the same temperature (Figs. 2*B* and 2*B*′). At probe temperature, 27.6°C, the line continued to sharpen slightly over a period of an hour or longer in a similar way to the water sample. The xenon samples were always broader, and of a different shape than the pure H_2O.

The maximum pressure within the capsule, if all the xenon were in the gas phase, was 28 atm. Results on relaxation times of protons in water at high pressures[17] indicate no effect below 4000 atm, and hence broadening or other effects due to this source have been ruled out.

Discussion.—In the absence of exchange broadening $1/T_2 = 1/T_1$. If the correction for this broadening is made, then $[1/T_2]_{\text{corr.}} = 1/T_1$. The relaxation times $T_{2\text{corr.}}$ and T_1 for O^{17} are governed by the relaxation time of the nuclear electric quadrupole moment. All other mechanisms of nuclear relaxation are small compared to the quadrupole relaxation. If the motional narrowing approximation holds, then[18]

$$\left[\frac{1}{T_2}\right]_{\text{corr.}} = \frac{1}{T_1} = \frac{3}{125}\left(1 + \frac{A^3}{3}\right)\left(\frac{e^2qQ}{\hbar}\right)^2 \tau_{qr}. \tag{1}$$

Here A is the molecular asymmetry parameter, $\frac{e^2qQ}{\hbar}$ is the quadrupole coupling constant, and τ_{qr} is the correlation time characterizing reorientations of the molecule or molecular complex containing the nucleus. It is the duration of time between random rotational reorientations of the electronic surroundings of the nucleus.

The quantities A and $\frac{e^2qQ}{\hbar}$ have been measured for H_2O from vapor phase microwave experiments. The values are:[19]

$$A = 0.7 \pm 0.1$$

$$\frac{1}{2\pi}\frac{e^2qQ}{\hbar} = -8.1\ MHz \quad (5.1 \cdot 10^7 \text{ radians/sec}).$$

The motional narrowing approximation requires that

$$\left(\frac{e^2qQ}{\hbar}\right)\tau_{qr} \ll 1.$$

All possible physical models for water require that $\tau_{qr} \ll 10^{-10}$, and thus this approximation is justified.

Inserting the values for H_2O^{17} into (1) results in

$$(\Delta H)_{m.s.} \propto [1/T_2]_{\text{corr.}} = 1/T_1 = 72.0 \cdot 10^{12}\tau_{qr}. \tag{2}$$

The importance of this equation lies in the fact that it provides a means of determining the rotational correlation times for H_2O molecules in water and aqueous solutions quite uniquely from line width measurements. The rotational correlation time is of vital importance in a consideration of the structure of water, as will be seen from the following discussion. This type of equation has been used in studies of the quadrupole governed relaxation times of Cl^{35} and N^{14} in organic compounds.[20, 21]

Using the value for $[1/T_2]_{\text{corr.}}$ for water at 25°C from the *Results* section above and equation (2) results in the following value for the rotational correlation time:

$$\tau_{qr} = 2.7 \cdot 10^{-12} \text{ sec.}$$

This value compares very well with that determined from dielectric relaxation experiments at microwave frequencies.[22]

In the Bloembergen, Purcell, and Pound (BPP) theory of nuclear magnetic relaxation,[22] a structureless liquid consisting of spherical molecules of radii, a, containing the relaxing nuclei is assumed. Upon this assumption a rotational correlation time for nuclei in such a liquid may be derived. If the viscosity of the liquid is η and the temperature is T, this correlation time is

$$\tau = \frac{4\pi\eta a^3}{3kT}. \tag{3}$$

The interaction radius of the water molecule has been determined by several methods[4, 23] to be over $2.0 \cdot 10^{-8}$ cm. Using this value, it is found from equation (3) with $\eta = 0.01$ that the rotational correlation time for the structureless liquid should be $8.3 \cdot 10^{-12}$ sec. Thus, the observed time is smaller than the theoretical value by a factor of 3.

The fact that makes this disagreement especially significant is that a similar one has been observed for several liquids.[20, 21] It has been shown that the discrepancy may be removed by a theory[20] which derives a correlation time on the basis of the structural quasi-lattice model of liquids. In this model a molecule does not undergo rotational reorientation when it is situated at a lattice site. However, the theory assumes rotational reorientation when a molecule has sufficient activation energy to jump to the top of the energy boundary of its lattice cell. This energy is less than the energy required to escape from the cell and enter a nearby vacant lattice site since the latter process requires energy for diffusion.

Thus, the results of the experiments reported here on the quadrupole relaxation of H_2O^{17} at temperatures between 4 and 30°C fit well into the quasi-lattice model of the structure of water. The rotational correlation time determined from line widths of the O^{17} resonance serve as a measure of this structure.

At temperatures above 30°C a transition has been observed in liquid water by several methods including accurate heat capacity measurements[24] and effects on ionization of solutes.[25] At the present time the results of these O^{17} NMR experiments indicate strongly that there is an abrupt change in the rotational reorientation time and therefore in the structure of the liquid at about this temperature.

It is interesting that this transition takes place at physiological temperatures. Any addition to water at these temperatures leading to ordering of the liquid structure must therefore have a profound effect on the mechanisms of reactions involving water as a physical entity.

The interpretation of the complex spectrum below 4°C is hampered by the fact that the two published values for the H^1-O^{17} spin-spin coupling constant,[26, 27] 73.5 *Hz* and 113.5 *Hz*, differ significantly. Spectrum *B* of Figure 1 has underneath it an indication of the positions of the lines in the *absorption* spectrum assuming a spin-spin coupling constant of 73.5 *Hz*. It is likely that the spectrum is an unresolved spin-spin triplet, but more accurate analysis must await an accurate determination of the coupling constant.

The time delay effect in water mentioned in the paragraphs above has been observed before in connection with static magnetic susceptibility measurements[28] and other physical investigations,[4] but the observation has never been followed up. Also, the adiabatic compressibility of water seems to exhibit a hysteresis.[29] These effects indicate that there is a finite time required for the water quasi-lattice structure to come to equilibrium following a disruption of this structure. This phenomenon must be of biological importance. More accurate experiments are in progress on this point.

Xenon Hydrate.—The results of the xenon hydrate experiments may be easily fitted into the picture of the structure of water discussed above. In the solid hydrate it would appear as if the proper description of the individual water molecules is that they are in a very loosely ordered state, more or less free to undergo individual reorientation, as they are not in the very tightly bound lattice of ice. This picture is consistent with the X-ray crystal structure work on the hydrates,[11] and with the physical properties of the compound.

These experiments indicate that solvated xenon has a similar effect in that, compared to pure water, it increases the activation energy a water molecule must have in order to reach the lattice energy boundary and undergo a rotational reorientation. In essence, the xenon lowers the effective temperature of the water. The fact that these solutions do not appear to reach equilibrium rapidly is interpreted to mean that once inside the quasi-lattice polyhedra, activation energy is needed to break the bonds holding the lattice together in order to remove a xenon atom from solution.

The biological effect of xenon has been demonstrated recently[30] in another connection where it was shown that the presence of a hemoglobin-xenon complex resulted in an increase in protein bound water.

Further experimental work on the biological implications of the effect of solutes on the structure of water using the techniques described above are in progress in this laboratory.

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