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Proton spin lattice relaxation times in biological tissues
Water and lipid role

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The multiphase model of water present in biological tissues derives mainly from the N.M.R. observation of a fraction of non-freezing water in biological tissues which is attributable to the water bound to macromolecules. Studies of this problem rule out completely the lipids as the source of the NMR signal. Our studies on nuclear spin lattice relaxation times of human and animal tissues have been made to understand if other contributions to the N.M.R. signal are present in addition to that coming from the water protons. We have measured directly by the N.M.R. method the relative water and lipid content and the relaxation time T_1 as a function of the water content which was varied by controlled dehydration. The results show clearly that lipids contribute actively to the N.M.R. signal and the fast relaxation time T_1 which is of the order of 100 ms in all biological tissues is related to the lipids. In view of these experimental observations we think that it is opportune to reconsider critically all the determinations of the 'bound water' made by the freezing procedure with the N.M.R. technique, and dedicate more attention to the lipids of biological membranes.

1. General aspects

Different models have been suggested for the behaviour of water in biological systems on the basis of proton nuclear relaxation studies. The main theories are based on the idea that water in biological tissues consists of ordinary water with a small fraction bound to proteins of other hydrophylic sites. Others consider that there are at least three fractions of water with a slow exchange between the hydration water of the protein and the mioplasmic water [1].

Because of the sensitivity of N.M.R. to the molecular dynamics, intensive use of this technique has been made in order to understand the role of water in biological systems. We have investigated the problem of the existence of bound water, giving attention to lipids as the source of the N.M.R. signal in dehydrated samples. The main problem in this kind of study is not only to determine the water and lipid content in the samples accurately, but also to use the same sample for the determination of T_1 and the water and lipid content and to measure directly in it the T_1 value of the lipids. With few exceptions [2, 3], the relation between T_1 and the water content has been made by referring to different animal or human tissues with intrinsically different

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water and lipid contents [4–10]. This kind of approach to the problem, as we shall see, is not correct because of the specific T_1 versus water content for each tissue.

2. Experimental and results

The human breast samples studied by N.M.R. were part of specimens surgically excised and histologically controlled for diagnostic purpose. The cases defined as normal refer to patients without breast pathology. The T_1 measurements were made using a Bruker SXP 2-100 spectrometer at a working frequency of 21 MHz (0·5 T) with a 180°–t–90°–τ–180° sequence at a temperature of 37°C, controlled by Bruker BVT-1000 temperature control unit. The samples, with a volume of the order of 0·1 cm^3 and without particular manipulation, were packed gently by centrifugation in a N.M.R. tube with an internal diameter of 6 mm and sealed with tape to avoid evaporation during the measurements. The T_1 measurements began about 1 hour from excision. The signal, recorded in phase sensitive mode, was digitized by a Datalab DL 912 transient recorder and sent for averaging and data analysis to an on-line Personal Computer Olivetti M24. The field stability, essential for phase sensitive detection, was assured by a Bruker B-R20 regulation unit. Under the working hypothesis that the N.M.R. signal comes from two components, water and lipids, we fitted the experimental points of the inversion recovery curve with two exponentials. For all human breast tissue samples examined, we found the shortest relaxation time T_{1f} to have a value in the range 100–200 ms. The longest relaxation time obtained, T_{1s}, shows a significant difference in values between normal and pathological tissues and also for different breast pathologies.

Before starting the T_1 measurement the free induction decay (FID) amplitude, At, due to all protons of the sample, was accurately determined. After determination of T_1, at a controlled temperature of 37°C, without moving the sample from the N.M.R. probe head, the sample was connected to a vacuum system (rotative plus diffusive vacuum pump) and pumping started. During the pumping the FID was monitored at fixed time intervals in order to follow the dynamics of the water extraction; a typical signal intensity versus time curve is shown in figure 1. The pumping continued until no further signal variation was observed. We should like to stress here that during the pumping action the sample was held at a constant temperature of 37°C in order to

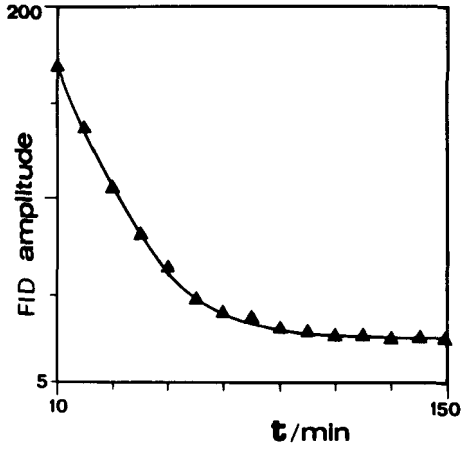

Figure 1. Free induction decay amplitude versus time during the dehydration process.

avoid the water from freezing due to rapid evaporation. The minimum pressure was 1–10 mPa. Following our working hypothesis, the FID amplitude Af, measured at the end of water extraction, is related to macromolecules, lipids and proteins present in the tissue. This residual signal is generally very small and the T_1 measurements are not very accurate. However, we have measured the relaxation time T_1 for this residual tissue component obtaining a mean value for $(T_1)_r$ of 91 $\pm$ 22 ms. Because of the poor accuracy of the measurements, we reject any effort to separate lipid and protein T_1 by a computation technique. Moreover, the protein signal is expected to be solid-like and because of the dead time of the receiver (40–50 μs) we expect that it will not contribute significantly to Af. The validity of this point of view has been subsequently confirmed experimentally and will be discussed later in the paper. From a knowledge of At and Af, the relative percentage of water was determined for the human normal and pathological breast tissues, with the respective T_1s values. We checked the validity of the method followed for water and lipid determination by the simultaneous use of a standard method on animal samples with an initial different content of water and lipids. The results reported in figures 2 and 3, show excellent agreement. What is particularly significant is the agreement of the two methods for the lipid determination. In fact it is an experimental demonstration that, after water

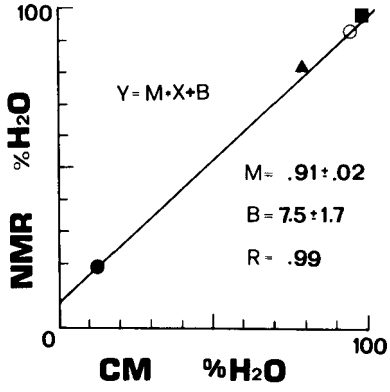

Figure 2. Comparison of the relative water percentage determined by the N.M.R. method (1-Af/At) with that obtained by the conventional method (CM), for different animal samples: ■, bovine muscle; ○, rabbit liver; ▲, bovine spinal marrow; ●, bovine breast.

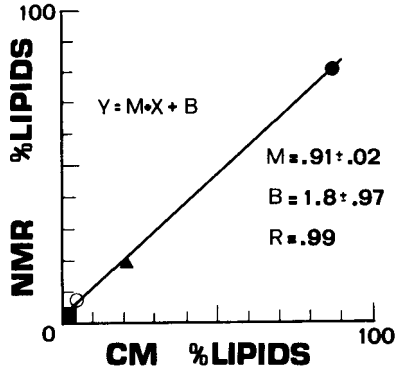

Figure 3. Comparison of the relative lipid content determined by the N.M.R. method with that obtained by a chemical procedure (the samples are the same of that for figure 2).

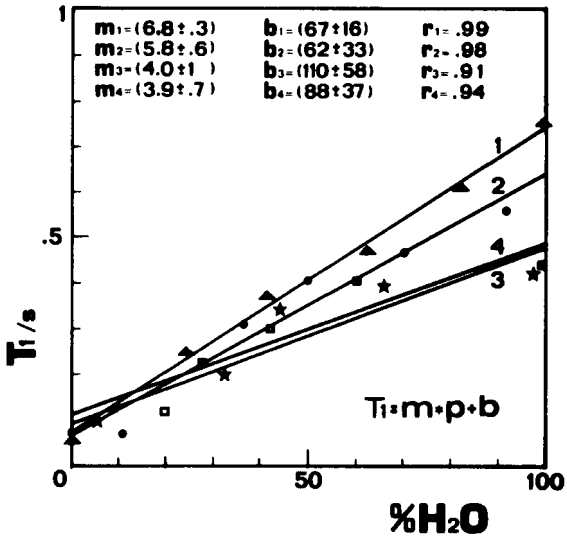

Figure 4. Apparent T_1 versus water content varied by controlled dehydration for different animal samples: ▲, bovine muscle (1); ●, bovine white matter (2); ★, chicken liver (3); ■, bovine breast (4).

extraction, the residual N.M.R. signal Af is due only to lipids. Consequently our fundamental working hypothesis of only two sources for the N.M.R. signal in biological tissue, water and lipids is correct. With these experimental results we planned to measure in some animal tissues (bovine breast, bovine muscle, chicken liver and bovine white matter) the relaxation time T_1 for different water contents varied by controlled dehydration. In this way it was possible to measure T_1 as a function of the water content in each tissue. The results reported in figure 4 show linear behaviour with a different slope for each tissue. Particularly interesting is that the extrapolation to zero water content gives, within experimental error, for all the cases examined a T_1 of the order of 100 ms, with a mean value $(T_1)_o$ of 82 ± 22 ms. This value is in good agreement with $(T_1)_r$ values determined experimentally in biological tissues. In view of these results and on the basis of the experimental

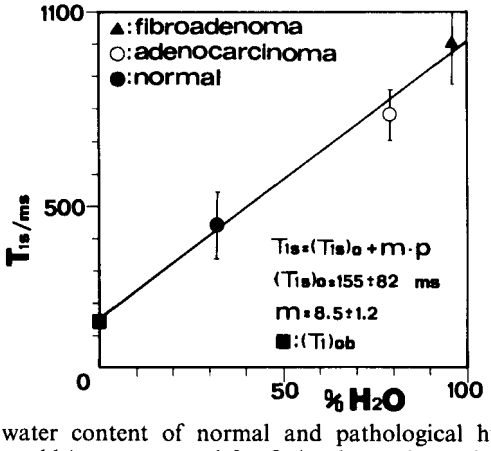

Figure 5. T_{1s} versus water content of normal and pathological human breast samples. ■,: $(T_1)_{ob}$ (see the table) was not used for fitting but only to show the agreement with $(T_{1s})_o$.

observation that human breast tissue shows a water content depending on its pathology, we fitted the T_{1s} determined for different human breast diseases as a function of the water content, obtaining the straight line shown in figure 5, with an intercept at zero water content corresponding to $(T_{1s})_o$ of 155 $\pm$ 89 ms. This value is in good agreement with that obtained in the case of T_1 measurements as a function of the water content for a human breast sample giving $(T_1)_{ob}$ of 138 $\pm$ 44 ms, and also with the mean value of the shortest relaxation time T_{1f} of 152 $\pm$ 67 ms, obtained by separating with a computation technique the two contributions to relaxation for all human breast samples examined.

3. Discussion

In the last decade many papers have been published on the role and behaviour of water in biological tissues. This interest has increased since the advent of the N.M.R. imaging technique for which tissue characterization by N.M.R. parameters is essential. Theories were tentatively formed in order to explain the increased relaxation times, T_1 and T_2, observed in pathological tissues. The analysis of these results is very difficult because of the varying working frequency, temperature, sample handling and experimental N.M.R. procedures. The present work was planned to have, independently and homogeneously, experimental data relating to T_1 of human breast tissue with different pathologies at 37°C and a field of 0·5 T, which is, at the moment, the working field for the majority of N.M.R. body imaging systems for routine diagnostic purposes. For the same reasons we have studied the water and lipid content of this tissue in order to understand if and which relation exists between them and the increase in T_1. Existing models are mainly concerned with the multiphase system of the water present in the tissues, based on two types of water: free water with a behaviour typical of bulk water, and water structured or bound to macromolecules via hydrogen bonds. This view is supported by experimental observations made by N.M.R. [9, 11–18]. However, in our opinion, this method may give same erroneus quantitative results because of the supercooling phenomenon which may occur when the system is cooled slowly, as happens in the N.M.R. sample. This non-frozen water is generally recognized as bound water which is responsible for the N.M.R. signal at a temperature well below the freezing point. We think that more experimental controls are needed to be sure that this supercooling effect is not present. Moreover, reducing the sample temperature to very low values will produce a reduction of the diffusion effect and consequently the water molecules will spend more time near the macromolecules. Thus, the quantity of the hypothetical bound water may be increased by experimental conditions. The studies based on this approach rule out completely the lipids as the source of the N.M.R. signal. Our results show clearly that dehydration by pumping action at constant temperature, if done carefully, will extract the water completely. Measuring the relaxation time in the residual part of these samples, after dehydration, we obtain a mean value $(T_1)_r$, which within experimental error, is the extrapolated value at zero water content in different tissues $(T_1)_o$ = 82 $\pm$ 22 ms (see figure 4). In order to check this last point, we have measured the spin–lattice relaxation time T_1 under the same experimental conditions 0·5 T and 37°C) of a pure lipid in its powdered form (*L*-α-phosphatidylcholine dimyristoyl supplied by the Sigma Chemical Co.); this is one of the components of biological membranes and fat. The N.M.R. signal in this lipid is small, but sufficient to permit the measurement of (T_1) to give a value of 94 $\pm$ 5 ms. This is of the same

The spin–lattice relaxation times.

T_{1f}/ms	$(T_{1s})_o$/ms	$(T_1)_{ob}$/ms	$(T_1)_r$/ms	$(T_1)_o$/ms	$(T_1)_L$/ms
152 ± 67	155 ± 89	138 ± 44	91 ± 22	82 ± 22	94 ± 5

T_{1f}, mean value of the fast relaxation component of a human breast sample, obtained by computation; $(T_{1s})_o$, extrapolation to zero water content of the slow relaxing component used for breast tissue characterization (see figure 4); $(T_1)_{ob}$, T_1 value extrapolated to zero water content from the relaxation measurement versus the water content for a human breast sample; $(T_1)_r$, mean value of the relaxation time measured on the sample after dehydration; $(T_1)_o$, mean value of the relaxation time extrapolated to zero water content for different animal tissues (see figure 5); $(T_1)_L$, spin–lattice relaxation time of the pure lipid determined at 0·5 T and 37°C.

order as that reported in the literature for bound water in biological tissues [2, 14–17, 19–21]. All the T_1 values referred to in our discussion are reported in the table. In view of these experimental observations, we think that it is opportune to reconsider critically all the determinations of bound water made by the freezing procedure using the N.M.R. technique, and to devote more attention to lipids present in biological tissues. Obviously the water and lipids are not independent systems and the molecular interactions existing between them should be investigated more thoroughly in order to understand the modifications of the spin–lattice relaxation time in pathological tissues.

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