Proton NMR-Relaxation Dispersion in Meconium Solutions and Healthy Amniotic Fluid: Possible Applications to Medical Diagnosis

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In order to show that for a possible application in medical diagnosis NMR-relaxation experiments at low Larmor frequencies ($\nu_0 \leq 20$ kHz) are more sensitive than the up to now done high field measurements in the MHz-range, we present dispersion curves ($\nu_0 = 50$ Hz to 50 MHz) of the proton longitudinal relaxation time T_1 and values of the transversal relaxation time T_2 for the example of amniotic fluids. Only for Larmor frequencies below $\simeq 100$ kHz the relaxation times for healthy amniotic fluid and pathological meconium solutions are significantly different, whereas at high Larmor frequencies, *i.e.* in the conventional MHz-range, the observed changes are rather small.

Introduction

The importance of a determination of proton relaxation times in biological water, in view of medical diagnosis, has been evident since the pioneering works of R. Damadian [1]. The nonperturbative character of this technique (if compared to X-Rays diagnosis methods) and the possibility of applying it *in situ* [2] have motivated a large number of works. Unfortunately, the effects observed so far are rather small and open to discussion. It will be demonstrated in the present paper that the diagnostic capability can be appreciably improved by making the relaxation measurements at Larmor frequencies (ν_0) in the kHz range instead of employing MHz frequencies, accessible to conventional NMR spectrometers. The essential point of our study is the well-established fact of relaxation spectroscopy [3] that the proton relaxation rate of aqueous macromolecular solutions strongly increases with decreasing values of ν_0.

As shown in the following, the study of the dispersion of the longitudinal proton relaxation time T_1 of a physiological fluid, over a large range of proton Larmor frequencies ν_0, has permitted to find the most favourable frequency regime to distinguish unambiguously between healthy and pathological tissues by relaxation time measurements. The example chosen in our study is the pollution of the amniotic fluid (AF) by meconium, normally eliminated by new borns in the hours following the birth. In case of foetal distress, happening during the last weeks of the pregnancy, the meconium is emitted early by the foetus, and the amniotic fluid gets polluted. If this is the case, an early delivery is necessary [4]. We considered this problem for three reasons:

(a) It is important for the physician to be informed about the pollution of the AF by the meconium.
(b) The techniques presently employed to control this pollution are not satisfying: amnioscopy [5], often badly tolerated, is quite imprecise; amniocentesis [6], more precise, is not without danger.
(c) The choice of a physiological fluid, bulky, well localized and with a well-defined composition, gives excellent conditions for analysis and interpretation of the experimental results.

In this work, we compare, at similar concentration of macromolecules (some g/l), the T_1 dispersion curves for the water protons in normal (healthy) amniotic fluid (AF) and pathological meconium

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solutions (MS). The measurements have been done over a very large proton Larmor frequency range, covering a variation of more than 6 orders of magnitude. The results obtained allow a comparison of healthy AF and meconium polluted AF. Our work completes and extends an already published short communication on this problem [7].

Experimental Techniques and Results

Experimental techniques

The dispersion of the longitudinal relaxation time T_1 has been measured between 20 Hz and 50 MHz (V. G. and F. N.) with the field cycling technique [3]. This technique permits the determination of the decay of the NMR signal at a fixed and relatively high frequency $\nu_{0\max}$ (or magnetic field) resulting from the effect of the relaxation for frequencies of arbitrary value between a few Hertz and $\nu_{0\max}$ in the Megahertz regime. The transversal relaxation time T_2 was determined (G. B., B. B., E. H., and P. M.) in the earth's magnetic field ($\nu_0 \simeq 2$ kHz) after prepolarization in a higher field ($\sim 5 \cdot 10^{-3}$ *T*) for signal enhancement [8], and at $\nu_0 = 100$ MHz by means of conventional pulse techniques [3]. The homogeneity and the constancy of the earth's field in the measuring station lead to a direct determination of T_2 from the exponential envelope of the free precession signal [9]. Both T_1 and T_2 have been studied at 37 °C with an accuracy of ± 3%. The sample volume necessary for our field cycling technique is about 1.5 cm³ [3].

Samples

The normal amniotic fluid (AF)* (10) has, during the last weeks of the pregnancy, a rather simple and constant composition: Except for some products in suspension, it is an isotonic solution of mineral salts (about 9 g/l, mainly NaCl), with a small protein concentration (about 2 g/l). These proteins are mainly those of the human blood serum in similar proportions. It is assumed that other components, which can be present in low concentration, do not participate actively in the relaxation mechanisms.

The meconium solutions (MS) have been prepared** as two different solutions of meconium (MS1:2 g/l; MS2:7 g/l) in isotonic liquid (water + 9 g/l NaCl). The composition of the meconium is complex and not constant: It contains mainly mucopolysaccharides (80% or more), pigments and salts which give it its colour, and other products which are not soluble (foetal hair, squamous cells, etc.) [11].

The main reason for comparing the normal amniotic fluid with meconium in isotonic solutions, instead of using amniotic solutions, is to identify more clearly the effect of the meconium on the proton spin relaxation times T_1 and T_2 in these systems.

Results

As illustrated in Fig. 1, both the MS solution and the AF show a rather complicated T_1 and T_2 relaxation dispersion. The most important finding is that the behaviour of MS1 and MS2 is quite different from that of AF. Whereas the low frequency T_1

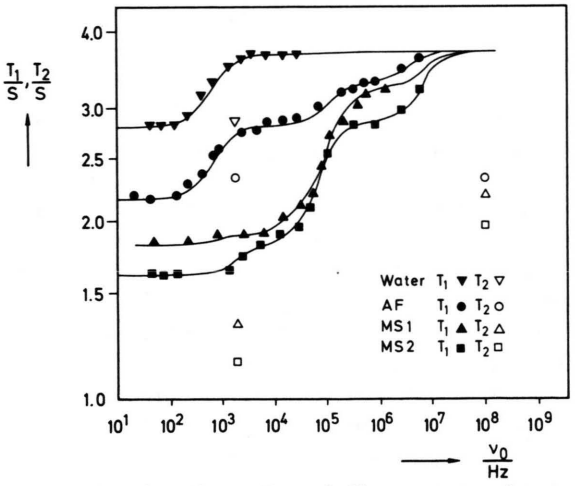

Fig. 1. Relaxation times T_1 and T_2 *vs.* proton Larmor frequency of investigated fluids (AF, MS) at 37 °C. For comparison, the T_1 dispersion of pure water [12] is included. Curves: Computer fits of the three-phase model [14] extended by proton exchange in free water [12] for the low frequency dispersion. The fitted model parameters (τ_i, N_i) are listed in Table I.

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dispersion of AF resembles to the one observed in pure water [12], which is plotted in the figure for comparison, this is not the case for MS. The high frequency T_1 dispersion data for MS 1, MS 2, and AF are all very similar. For all measured frequencies, *i.e.* between 20 Hz and 50 MHz, T_1 of the normal healthy fluid (AF) is longer than T_1 of the polluted MS samples with a maximum difference of about 60% in the 10 kHz-range. For comparison, in the high frequency range (1 – 10 MHz) the difference in T_1 amounts to only 10 – 15%. In spite of the restricted T_2 data the effect of meconium is also revealed in this case. Obviously, the T_2's for MS are strongly frequency dependent, whereas the T_2's of AF are not. Note that for AF the low field T_2 approaches the T_1 relaxation rate, whilst the T_2 of MS in the same frequency range is still appreciably shorter than T_1.

Discussion

Diagnostic capability

Biological tissues and physiological fluids are known to possess slow molecular motions with long correlation times τ_c. Therefore, one understands why a comparison of NMR-relaxation times (T_1 or T_2) of healthy and, for example, cancerous tissues with conventional NMR-spectrometers, which operate at Larmor frequencies ν_0 in the MHz-range where T_1 or T_2 are relatively insensitive to reorientations with long correlation times ($\tau_c > \nu_0^{-1}$), does not allow a sufficiently clear identification of these two cases [2]. As shown in Fig. 1, the ratio of the T_1 values for AF and MS 1 or MS 2 at 37 °C under conventional conditions is always smaller 1.2; therefore, measurements of T_1 at high Larmor frequencies do not give the best conditions to make clear whether there is meconium present or not.

However, our relaxation dispersion measurements clearly demonstrate that for both relaxation times T_1 and T_2 the capability to distinguish between healthy and pathological fluids increases by using Larmor frequencies below about 100 kHz. In this low frequency range ($\tau_c < \nu_0^{-1}$) the T_1 and T_2 of MS 1 or MS 2 is about 60% shorter than the T_1 or T_2 of AF. Similarly, the differences in T_2 for AF and MS solutions become larger for smaller Larmor frequencies. In addition to that, another distinction between AF and MS is possible by the evaluation of the low frequency T_1/T_2-ratio. For pure water, as well as for normal AF, the value of T_2 differs only slightly from the value of the corresponding T_1 at the lowest Larmor frequencies ν_0 explored. This result agrees with the theoretical concept that for such fluids there exists no very slow molecular motion with a correlation time long enough to realize the regime $\tau_c > \nu_0^{-1}$ at frequencies smaller than 100 Hz. However, in the case of meconium solution MS 1 and MS 2, T_2 as measured at $\nu_0 \simeq 2$ kHz (earth's field), is appreciably smaller than T_1 even at the lowest studied frequency. More precisely, for normal AF (and water) on finds T_1 (25 Hz) $\simeq T_2$ (2 kHz) while, for meconium solutions (MS 1 and MS 2) T_1 (50 Hz) $\simeq 1.4\, T_2$ (2 kHz), which implies a very slow motion. Although it is presently difficult to give a reliable interpretation of this very slow molecular motion, it still constituents an excellent test to distinguish AF and MS.

The comparison of the water results with those of the other samples reveals the following: Assuming that the T_1 dispersion in pure water is close to that of isotonic liquids (IL), *i.e.* a diamagnetic salt solution (13), it is possible to separate various contributions to the relaxation rates in the studied samples (AF ≡ IL + 2 g/l proteins; MS 1 ≡ IL + 2 g/l meconium; MS 2 ≡ IL + 7 g/l meconium). The 4 different T_1-dispersion curves illustrate that each additional ingredient to the isotonic liquid decreases the relaxation times. Therefore, we expect to find smaller relaxation times for AF polluted by meconium (AFM) than for the MS samples, so that the diagnosis capability should become even better for AFM than the 60% change in T_1 described above.

Relaxation model

Now, in order to find out the differences between healthy AF and pathological MS more quantitatively, we fitted our T_1 relaxation dispersion data to a biological water model developed previously [14], extended by the frequency dependence of pure water due to proton exchange [12]. This model is based on a "three-phase" description of H_2O molecules in biological systems, in which each phase is characterized by its own type of motion with distinctive reorientation times. Following this idea, water in such systems consists of three types of environments, namely:

Table I. Model parameters (reorientation times τ_i, proton concentrations in units of pure water proton concentration N_0) of the studied samples, evaluated by computer fit of the experimental T_1 data (Fig. 1) to the extended three-phase model [12, 14]. Note that $N_1 + N_2 + N_2' + N_3 = 1$.

	Irrotational H_2O		Hindered H_2O				Free H_2O		
Sample	τ_1 [s]	N_1 [N_0]	τ_2 [s]	N_2/N_1	τ_2' [s]	N_2'/N_1	τ_3 [s]	τ_e [s]	N_1 [N_0]
H_2O	–	–	–	–	–	–	–	2.6×10^{-4}	1
AF	0.7×10^{-6}	3.2×10^{-3}	1.3×10^{-8}	0.23	7.0×10^{-11}	≈ 5	–	2.4×10^{-4}	≈ 0.98
MS 1	1.1×10^{-6}	1.4×10^{-3}	0.4×10^{-8}	0.11	2.5×10^{-11}	≈ 5	–	1.9×10^{-4}	≈ 0.99
MS 2	1.5×10^{-6}	2.5×10^{-3}	1.0×10^{-8}	0.15	4.0×10^{-11}	≈ 5	–	1.0×10^{-4}	≈ 0.98

(a) H_2O non-rotationally bound to macromolecules (reorientation time τ_1, proton concentration N_1);

(b) hydrated H_2O, comprising both rotationally bound (reorientation time τ_2, proton concentration N_2) and translationally hindered (reorientation time τ_2', proton concentration $N_2' \gg N_2$) molecules, and

(c) free bulk water (reorientation time τ_3, proton exchange time τ_e, proton concentration N_3).

The curves in Fig. 1 show the computerfit of the model to the experimental data with the obtained τ's and N's listed in Table I. Note that the value of τ_3 is not accessible because of the high-frequency limit of the measurements, *i.e.* τ_3 is too small to give a dispersion step in the studied frequency range. We see that both the various reorientation times and the concentrations are very similar for the AF and MS samples, or even equal within the experimental error limits. This is not surprising, since the T_1 dispersion steps occur at nearly the same frequency, and the maximum T_1 change amounts to only 60%. However, Table I reveals some trends for the variations of τ and N which are quite reasonable.

E.g. (a) we notice a monotonic decrease of τ_e in the order: pure water, healthy AF, MS 1 (2 g/l) and MS 2 (7 g/l). We can correlate these results with those of Migchelsen and Berendsen (15) who have shown that values of this constant are quite dependent on the polyionic character of present macromolecules. Moreover, the polyionic character of muco-polysaccharides is much more pronounced than that of proteins in the AF. Also it is well-known that τ_e is very sensitive on the pH of the solution, any deviation from pH = 7 shortening the exchange time. (b) For the MS samples the other time constants (τ_1, τ_2, τ_2') show the opposite behaviour, *i.e.* a slight increase in the order specified above. This reflects the fact that the mobility of hydrated water becomes more strongly hindered when the concentration of macromolecules becomes greater. (c) As to be expected, N_1 varies approximately proportional to the meconium concentration, whereas N_2/N_1, N_2/N_1 and N_3 are nearly constant within the error limits. This observation parallels results obtained with protein solutions [14] and, most likely, is a consequence of the restricted number of charged and dipolar sites on the macromolecules. (d) Finally one may wonder why none of the τ's reflects the enormous difference of molecular weight (factor 10^5) between the solutes in AF and MS. This finding probably indicates, as do the low T_2 values for the MS samples, the existence of a very low frequency T_1 dispersion, which was not observable with our field cycling techniques.

Possible applications

As we have shown, the measurement of T_1 at Larmor frequencies smaller than 20 kHz, or the measurement of T_2 at 2 kHz, or the evaluation of the low frequency T_1/T_2 ratio makes it possible to distinguish between the healthy AF and solutions, even dilute, of meconium. These methods are now used in view of (a) a substitution of the spectrophotometric analysis of meconium polluted AF in view of the determination of the content of the meconium [16], and (b) an application *in situ* on pregnant women in the last weeks of pregnancy [17].

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