FACTORS AFFECTING PROTON MAGNETIC RESONANCE LINEWIDTHS OF WATER IN SEVERAL RAT TISSUES

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1. Introduction

Studies of proton magnetic resonance (PMR) linewidths of water in whole tissue samples have been previously reported for erythrocytes, nerve tissue, fish muscle, frog muscle, and rat skeletal muscle [1—6]. In general, the full linewidths measured at the peak half-height are sufficiently narrow to obtain accurate measurements for a large portion of the water in the samples by high resolution techniques. Although a number of such studies have been reported, no extensive efforst have been made to determine what factors affect these linewidths. Consequently, interpretation of some of these data may have been somewhat premature. In this communication it will be shown that at least two factors, namely magnetic field strength and tissue water content, can have significant effects on these linewidths.

Under favorable conditions the PMR linewidth ($\Delta\upsilon$) of a liquid sample can be taken to be $1/\pi T_2^*$ where T_2^* is the apparent proton nuclear spin—spin relaxation time [7]. A variety of factors can cause the linewidths to be field (frequency) dependent. Magnetic inhomogeneity effects in solid tissues can cause T_2^* to be shorter than the true spin—spin relaxation time (T_2) and lead to field dependent linewidths [8]. Interaction of water molecules with slow moving macromolecules such as proteins can also cause observable field dependence of relaxation times [9, 10]. In addition, exchange of water molecules between sites having different chemical shifts can cause T_2 to be field dependent [11]. Thus the effect of magnetic field strength on the linewidths is considered. Two factors that can have effects on the linewidths at a given field strength are the concentrations of dissolved macromolecules [12] and paramagnetic metal ions [13]. Dissolved macromolecules cause the molecular correlation times of some of the water molecules to be increased resulting in a decrease in the water proton relaxation times. Unpaired electrons on paramagnetic ions produce local magnetic fields which cause the water proton relaxation times to be reduced. Consequently, at least three factors should be considered: magnetic field strength, concentration of dissolved macromolecules, and concentration of paramagnetic ions.

2. Materials and methods

Samples were taken of blood, liver, kidney, epidermis, and Dunning hepatomas from Fischer rats and squamous call carcinomas from an August rat killed by ether. Blood samples were obtained from the hepatic veins or arteries using a syringe rinsed with a heparin solution. A brass cork borer was used to take plugs of all tissues except epidermis. The latter samples were obtained by scraping pelts that had been previously shaved as described by Suntzeff and Carruthers [14]. The samples were packed into Wilmad 506—PP 5 mm o.d. NMR sample tubes using a syringe needle to allow air to escape while the tissue was being packed firmly into the tube with a stainless steel rod.

The linewidths were measured at 100.00 and 13.56 MHz using a Varian HA—100D—15 spectrometer by using two different radio frequency units, probes, and magnet current settings. For most of the HA—100D—15 spectra, the 5 mm o.d. sample tubes were used in a coaxial arrangement with a Varian 12 mm o.d. sample tube containing tetramethylsilane in carbon tetrachlo-

ride for a frequency lock signal. These values are the average of five measurements per sample and are reproducible to ±0.1 Hz. The epidermis linewidths at 13.56 MHz were obtained in the HR mode with sideband calibration using only 5 mm o.d. tubes and are reproducible to ±1 Hz. The 60 MHz spectra were obtained on a Hitachi–Perkin Elmer R–20 spectrometer with a calibrated sweep width using 5 mm o.d. tubes. These values are the averages of three measurements per sample and are reproducible to about ±1 Hz.

The water, iron, and copper measurements were performed in duplicate on each sample. The water content was determined by comparing the weight of a sample of whole tissue to the weight of the sample after drying for 12–14 hr at 110° C at reduced pressure. The iron and copper contents were determined by atomic absorption spectrometry by the method of Parker et al. [15]. The samples were run using a modified Varian Techtron A.A–4 atomic absorption spectrometer set at 2483.3 Å for iron and 3247.5 Å for copper with an acetylene–air flame. The readings were compared to those of standards prepared by dissolving Mallinkrodt A. R. iron wire and Matheson, Coleman and Bell A.C.S. R. copper foil in redistilled Mallinkrodt A. R. nitric acid. All glassware used in the metal analyses was soaked in redistilled nitric acid and double distilled water prior to use.

3. Results and discussion

The PMR linewidths of water in a variety of whole rat tissue samples were measured at two or three field strengths (operating frequencies). The linewidths appear to generally increase with increasing field strength (figs. 1a–c). An exchange broadening contribution to $\Delta\nu$ which depends on the square of the chemical shift difference between molecules in different sites is possible. Such a contribution would increase with increasing field strength. However, other workers have given evidence that this particular exchange effect is probably unimportant in similar systems [16, 17]. No new information on this possibility is offered here. It has been pointed out [8] that $1/T_2^* = (1/T_2) + \gamma/2(\Delta H)$, where γ is the magnetogyric ratio and ΔH, is the effective inhomogeneity of the magnetic field. If the main magnetic field, (H_0) is homogeneous, ΔH might

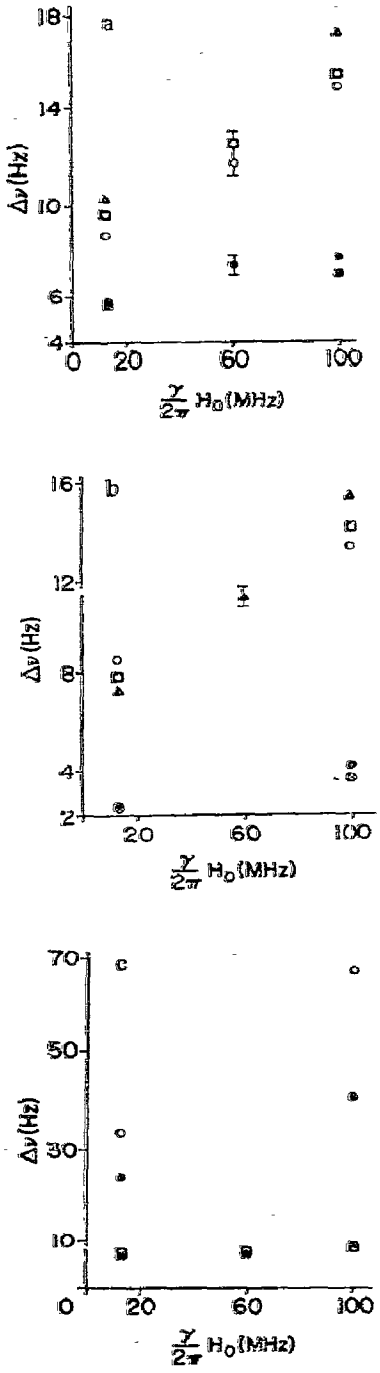

Fig. 1. Field dependence of linewidths: a) Upper three sets liver, lower two sets Dunning Hepatoma.
b) Upper three sets kidney; lower two sets blood.
c) Upper two sets epidermis; lower two sets squamous cell carcinoma.

be thought of as arising from the effective variation of the magnetic susceptibility (G_iX_i) throughout the sample. Then by analogy to treatments of the effects of magnetic susceptibility on chemical shifts [18], ΔH would be given approximately by $H_0\,(1-\Delta(G_iX_i))$. It should be pointed out that $1/T_2$ is also slightly field dependent [19], generally decreasing with increasing field strength. The changes in $\Delta\nu$ observed here with changes in H_0 are thus the sum of these effects. The observed field dependence combined with the observation that homogenization of the tissues in a decrease in both the linewidths and field dependence of the linewidths indicates that inhomogeneity effects are significant. This conclusion is consistent with the observations of Hansen [20] and of Cooke and Wien [21].

In dilute protein solutions the water proton spin–spin relaxation times follow the equation $1/T_2 = 1/T_{2w} + kc$, where T_{2w} is the relaxation time for distilled water, k is an empirical constant, and c is the weight fraction of the solution that is protein [12]. This relation arises due to restriction of the mobility of the water molecules by interaction with the proteins. Thus $1/T_2$ increases linearly with increasing per cent protein in solution and decreases linearly with increasing per cent water in solution. In tissue samples the per cent protein actually dissolved may be difficult to determine. However, the per cent water in the microscopic protein solutions in the tissues should increase with an increase in the water content of the tissue. In Figs. 2a and 2b it is seen that $\Delta\nu$ decreases with increasing per cent water in the tissues. It is concluded that the PMR linewidths of water in tissues behave in a manner consistent with the relaxation behavior of water protons in protein solutions.

The electron spin resonance (ESR) spectra of whole tissues show a variety of peaks, some of which vary in intensity with time and method of preparation [22]. Without concurrent ESR studies only rough estimates of the effects of paramagnetic metal ions on the water PMR linewidths of tissues can be made. One approach is to plot $\Delta\nu$ versus N, the number of possible paramagnetic metal ions/cc. [13] as shown in figs. 3a and 3b. N was calculated from the measured metal ion and water contents of the tissues. An apparent correlation is seen only for the squamous cell carcinoma and epidermis samples. This is probably due to coincidental differences in tissue water con-

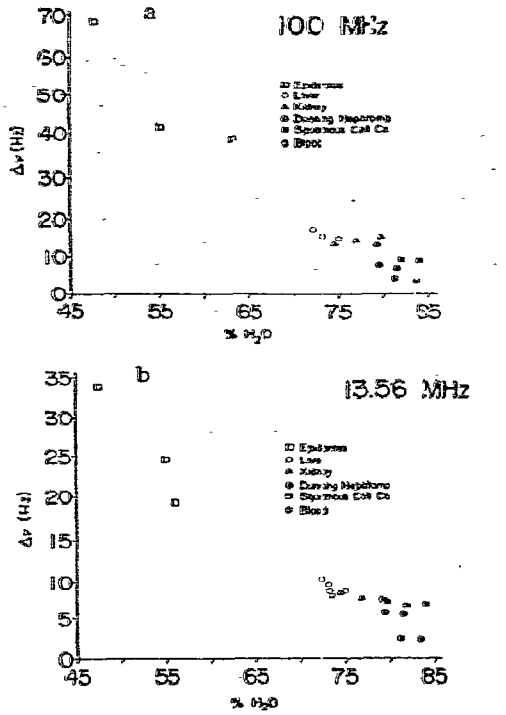

Fig. 2. Variation of linewidths with water content of tissues. a) At 100.00 MHz. b) At 13.56 MHz.

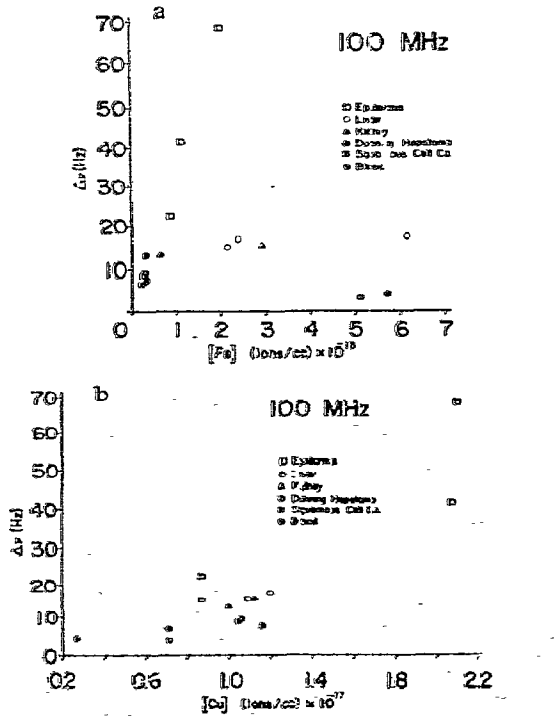

Fig. 3. Linewidths plotted versus concentration of metal ions. a) Fe. b) Cu.

tent, and not a true paramagnetic ion effect. This does not rule out the possibility of some paramagnetic ion effects, however, since there are multiple valence and spin states available to these ions. These plots are only suggestive that paramagnetic ions are probably not responsible for the predominant relaxation effects in the tissues.

Some comments should be made on the relevance of these findings to the pulsed PMR studies reported by Damadian [23] and by Hazelwood et al. [24] on the relaxation times of water in normal and tumor tissues. Their studies showed that T_2 values for water in some tumor tissues are longer than for water in the corresponding normal tissues. Damadian has suggested that the structure of water in tumor tissues is altered relative to the structure of water in normal tissues. The observation (figs. 2a, b) that $\Delta\nu$ decreases with the increasing water content of the tissues is suggestive that a more likely explanation for the differences in relaxation times involves the increased water content of tumor tissues relative to normal tissues. In addition, the fact that the tumor tissues showed consistently narrower linewidths than the corresponding normal tissues suggests that continuous wave PMR measurements should be considered as a means of distinguishing normal from tumor tissues. This possibility is currently being investigated.

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