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Tissue Water Content and Nuclear Magnetic Resonance in Normal and Tumor Tissues

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SUMMARY

Pulsed proton nuclear magnetic resonance was used to differentiate between normal and malignant tissues.

When the tissue water content varied from 80 to 93%, the tumors exhibited spin-lattice relaxation times (T_1) from 0.9 to 1.8 sec. We report also the results obtained on 9-day-old embryos and on liver, brain, and heart from 2-day-old rats. A good correlation between the spin-lattice (T_1) and spin-spin (T_2) relaxation times and the tissue water content was found for all tissues studied. The relaxation times T_1 and T_2 and water content in Walker 256 carcinoma and its lymph node metastasis were quite similar.

INTRODUCTION

The use of NMR[2] spectroscopy as both a diagnostic and a research tool in cancer has been investigated by several research groups.

Much work in connection with the state of water in normal and malignant tissue has been published in the last 3 years. It has been recognized that NMR is potentially a very powerful tool for investigating this type of problem, because it allows a single component to be studied in a heterogenous dispersion system.

Damadian (4) first used NMR techniques as a method for discriminating between tumor and normal tissue. The observed differences between the relaxation times of normal tissue and tumor tissue were interpreted as illustrating the degree of perturbation of endosolvent structure that can accompany malignant transformation; these differences also suggest a significant decrease in the degree of ordering of intracellular water in malignant tissue.

Hazlewood *et al.* (6) have measured the relaxation times and diffusion coefficient of water protons in normal and tumor tissue. They attributed the increase in the relaxation times for tumor tissue to a change in the interaction of water molecules with macromolecular structures of the cell. In another study, Hazlewood *et al.* (7) asserted that the genotype of the host influences the relaxation times.

We suggested (12) and then demonstrated (11, 14) that the differences in relaxation times T_1 and T_2 between neoplastic tissues and normal tissues are due to the greater tissue hydration in the neoplasm. Later, Inch *et al.* (10) and Saryan *et al.* (18) confirmed our original observation concerning T_1. Block (1) pointed out the effect of water content on T_2 relaxation times in several rat tissues.

This report presents spin-lattice (T_1) and spin-spin (T_2) relaxation times for 4 rat tumors, 2 mouse tumors, embryo, and 3 immature rat tissues. Water content of the tissues was also measured.

MATERIALS AND METHODS

Proton nuclear magnetic relaxation times were measured at room temperature (293° ± 1°K), at 60 MHz, using a standard NMR-pulsed Bruker N-KR 322-5 spectrometer. The longitudinal relaxation time, T_1, was measured by the null method (2). The transversal relaxation time was measured with the usual Carr-Purcell technique as modified by Meiboom and Gill (17). The 90° and 180° pulse widths were 2.5 and 5 μsec, respectively. The measurements were carried out only on the "slow" liquid-like part of the signal; the "fast" decaying portion was generally of negligible amplitude, so no attempt was made to study it.

Mature Wistar rats weighing 180 ± 20 g were used. T_1 and T_2 nuclear magnetic relaxation times and water content were measured for 4 transplantable tumors: Walker 256 carcinoma transplanted s.c. for 5 years; epithelioma T_8 Guérin transplanted for 6 years (from the Cancer Research Institute of Cluj, Cluj, Romania); H-18R; and R-20 (gift of Dr. I. Popp, Cancer Research Institute of Bucharest, Bucharest, Romania).

Eight- to 10-week-old RAP mice bearing Ehrlich-Lettré tetraploid ascites carcinoma and Ehrlich solid carcinoma were used.

All mice and rats were killed by cervical dislocation. The samples were placed unwashed into standard test tubes with an outside diameter of 8 mm for NMR analysis. The NMR measurements were obtained within 30 min of tissue removal.

Water content was determined on representative samples by weighing freshly excised tissue, drying it in an oven at 100° for 24 hr, and reweighing it.

RESULTS AND DISCUSSION

The dependence of water proton relaxation times T_1 and

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[2] The abbreviation used is: NMR, nuclear magnetic resonance.

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T_2 on water content of each tissue is shown in Table 1 and plotted in Charts 1 and 2.

A review of the relaxation mechanism that could explain the enhanced relaxation times T_1 and T_2 in tumor tissue as compared with normal ones has been published elsewhere (5, 8, 9). Another parameter that could influence only relaxation time T_2 is the tissue pH; this possibility has been presented previously (13, 15, 16). In this paper we discussed only the influence of water content in tumors on the relaxation times T_1 and T_2.

Clifford *et al.* (3) have shown that the results for systems containing a large proportion of water can be considered in terms of exchange between free and bound water.

If a fast proton exchange between free and bound water is assumed, then the observed (obs) relaxation time should be a weighted average of the relaxation rates of the free and bound water:

$$1/T_{obs} = (a/T_{bound}) + [(1 - a)/T_{free}] \quad (A)$$

where a is the fraction of bound water and $(1 - a)$ is the fraction of free water. Equation A may be written as:

$$1/T_{obs} = (1 - a)\,[(1/T_{free}) - (1/T_{bound})] + (1/T_{bound}) \quad (B)$$

Clifford *et al.* (3) studied human erythrocyte-water systems and reached 2 conclusions: (*a*) in samples with high water content (32 to 100%) the relaxation time T_1 is more sensitive to the modification of the free water fraction than of bound water; (*b*) in samples with lower water content (0 to 36%), relaxation time T_2 is more sensitive to the modification of the bound water fraction than is T_1. T_1 does not "feel" changes of the water content in this range.

Equation B explains the dependence of T_1 on tissue water content. The increase of free water shows a good correlation with the observed relaxation times T_1 of tissue water. The obtained values for the relaxation times in immature brain and heart have the same magnitude as those of malignant tumors tissues. This fact indicates that the observed differences between the relaxation times T_1 and T_2 of the malignant and normal tissues are not due to the increase in the freedom of tissue water molecules (4) or, to a lesser degree, of water structure in malignant tissue than in normal tissue (19).

There is a good correlation between the increase of T_1 and T_2 and the increase of water content in the immature tissues (liver, heart, and brain). The relaxation times T_1 and T_2 decreased as the tissues matured. The values of T_1 and T_2 in immature brain decreased from 1.361 and 0.149 sec, respectively, to 0.866 and 0.071 sec, respectively. The main cause is the decrease of the water content from 88.3 to 77%.

The same shortening of the relaxation times T_1 and T_2 with the maturation was observed for heart and liver while the water content decreased. Equation B shows that the term $(1 - a)(1/T_{free} - 1/T_{bound})$ is directly dependent on the free water fraction. The values of T_1, T_2, and water content of embryos are higher than those of malignant tissues.

Table 1

Spin-lattice (T_1) and spin-spin (T_2) relaxation times of tissue water.

Tissue	T_1 (sec)	T_2 (sec)	Water content (%)
Embryo	1.422 ± 0.007[a](5)[b]	0.200 ± 0.004 (5)	90.8 ± 0.5 (5)
Tumor tissue			
Ehrlich ascites (liquid + cells)	1.818 ± 0.016 (3)	0.226 ± 0.0052 (3)	93 ± 3 (3)
Ehrlich ascités (cells)	1.15 (2)		83 ± 0.8 (6)
Guérin	1.135 ± 0.012 (4)	0.059 ± 0.009 (4)	84.4 ± 2 (6)
H-18 R	1.125 (2)	0.080 (2)	
Walker 256	1.093 (2)	0.077 (2)	83.2 ± 0.47 (5)
R-20	1.072 (2)	0.080 (2)	
Ehrlich solid	1.025 ± 0.007 (3)	0.0382 ± 0.006 (3)	80.0 ± 1 (3)
Lymph node metastases of Walker carcinoma	0.916 ± 0.045 (3)	0.065 ± 0.006 (3)	83.7 ± 0.95 (3)
Immature tissue			
Brain	1.361 (2)	0.149 (2)	88.3 ± 0.87 (3)
Heart	0.936 (2)	0.069 (2)	86.6 ± 0.75 (3)
Liver	0.527 (2)	0.043 (2)	74.5 ± 0.66 (3)
Normal tissue			
Brain	0.866 ± 0.042 (3)	0.071 ± 0.007 (3)	77 ± 0.42 (3)
Heart	0.873 ± 0.027 (3)	0.046 ± 0.011 (3)	73.3 ± 0.39 (3)
Kidney	0.685 (2)	0.056 (2)	72.4 ± 0.58 (3)
Liver	0.340 (2)	0.030 (2)	65.8 ± 0.34 (3)
Distilled water	2.78	1.4	

[a] Mean values ± S.E.

[b] Numbers in parentheses, number of samples analyzed.

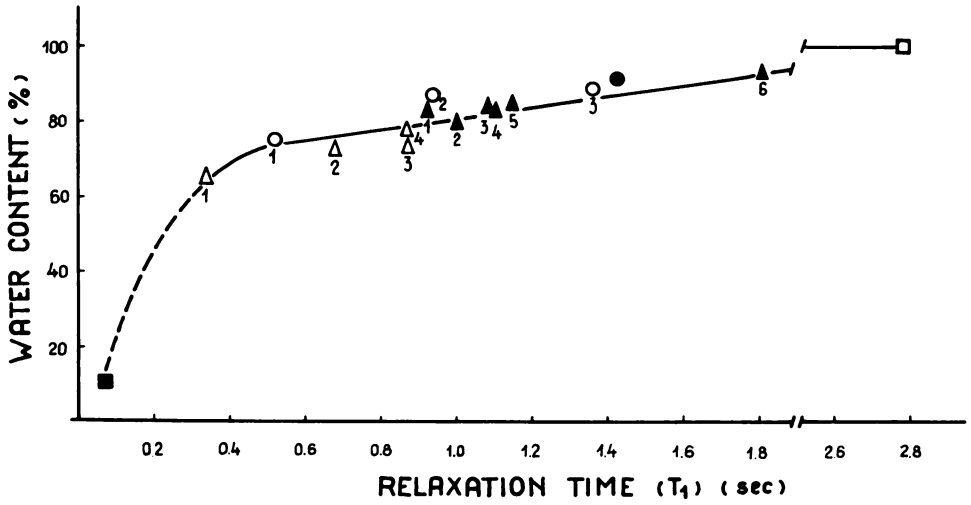

Chart 1. Percentage of water content *versus* T_1 relaxation time for various normal and malignant tissues. Each *point* represents the average of values for each tissue type. △, normal tissue: liver, (*1*), kidney (*2*), heart (*3*), and brain (*4*); ▲, malignant tissue: lymph node metastasis of Walker 256 carcinoma (*1*), Ehrlich solid tumor (*2*). Walker 256 carcinoma (*3*), Ehrlich ascites (cells) (*4*), Guérin T_8 (*5*), Ehrlich ascites (cells + liquid) (*6*); ○, immature tissue: liver (*1*), heart (*2*), brain (*3*); ●, embryo; □, distilled water; ■, dried spleen.

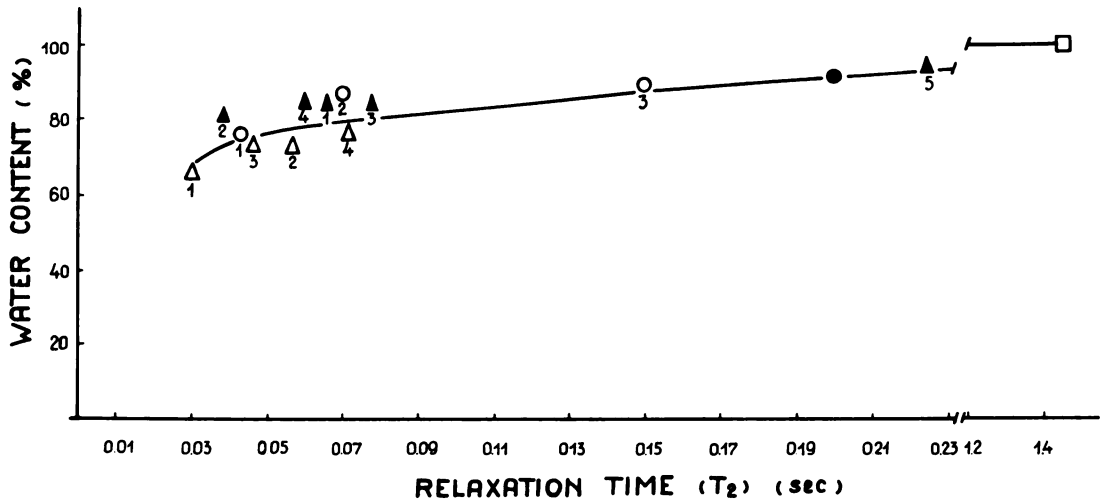

Chart 2. Percentage of water content *versus* T_2 relaxation time for various normal and malignant tissues. Each *point* represents the average of values for each tissue type. △, normal tissue: liver (*1*), kidney (*2*), heart (*3*), brain (*4*); ▲, malignant tissue: lymph node metastasis of Walker 256 carcinoma (*1*), Ehrlich solid (*2*), Walker 256 carcinoma (*3*), Guérin T_8 (*4*), and Ehrlich ascites (cells + liquid) (*5*); ○, immature tissue: liver (*1*), heart (*2*), and brain (*3*); ●, embryo; □, distilled water.

The observed differences between the relaxation times of the Walker carcinoma and lymph node metastases are not significant. More studies concerning the primary tumor and its metastasis should be undertaken before final conclusions may be drawn.

Our data on the normal and malignant tissues show a clear trend of increased water content associated with increased relaxation times. Consequently, the main cause of the differences observed between the relaxation times T_1 and T_2 of the normal and malignant tissue is the higher water content in the latter tissue.

From the results of this study we conclude that: (*a*) the main cause of the observed differences between the relaxation times T_1 and T_2 of the normal and malignant tissue is the greater tissue hydration in the neoplasms; (*b*) T_1 is more sensitive than T_2 to variations of tissue water content; and (*c*) the magnetic resonance relaxation techniques appear to be much less promising for detecting cancerous tissue than was originally thought. We suggest that pulse NMR techniques may not be able to produce meaningful results in this simple fashion, if only the water content is the principal cause.

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