SCIENCE

Tumor Detection by Nuclear Magnetic Resonance

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Abstract. *Spin echo nuclear magnetic resonance measurements may be used as a method for discriminating between malignant tumors and normal tissue. Measurements of spin-lattice (T_1) and spin-spin (T_2) magnetic relaxation times were made in six normal tissues in the rat (muscle, kidney, stomach, intestine, brain, and liver) and in two malignant solid tumors, Walker sarcoma and Novikoff hepatoma. Relaxation times for the two malignant tumors were distinctly outside the range of values for the normal tissues studied, an indication that the malignant tissues were characterized by an increase in the motional freedom of tissue water molecules. The possibility of using magnetic relaxation methods for rapid discrimination between benign and malignant surgical specimens has also been considered. Spin-lattice relaxation times for two benign fibroadenomas were distinct from those for both malignant tissues and were the same as those of muscle.*

At present, early detection of internal neoplasms is hampered by the relatively high permeability of many tumors to x-rays. In principle, nuclear magnetic resonance (NMR) techniques combine many of the desirable features of an external probe for the detection of internal cancer. Magnetic resonance measurements cause no obvious deleterious effects on biologic tissue (*1*), the incident radiation consisting of common radio frequencies at right angles to a static magnetic field. The detector is external to the sample, and the method permits one to resolve information emitted by the sample to atomic dimensions. Thus the spectroscopist has available for study a wide range of nuclei for evidence of deviant chemical behavior.

The resonance technique selected for this particular application belongs to a group of techniques known as "transient" or induction methods. In this experimental arrangement the sample continues to emit a radio-frequency signal for a brief but measurable period after the incident radiation (pulse) has been removed. This method makes possible the direct measurement of spin-lattice (T_1) and spin-spin (T_2) relaxation times, thus avoiding the uncertainties of estimating them from the line width measurements of steady-state NMR spectra. In addition, it also makes possible the characterization of biologic tissues on the basis of the properties of their emitted radio frequency.

In order to determine whether neo-plastic tissues could be recognized from their NMR signals, I studied the proton resonance emissions from cell water. Recent NMR work of Cope (*2*), Hazlewood *et al.* (*3*), and Bratton *et al.* (*4*) has provided fresh insight into the physical nature of cell water. These authors have independently concluded that the decreased NMR relaxation times observed for cell water relative to distilled water (Tables 1 and 2) are due to the existence of a highly ordered fraction of cell water in which the protons of the water molecules have correlation times substantially less than the Larmor period. The reduction of the correlation times is presumably due to the adsorption of water molecules at macromolecular interfaces, findings that are consistent with the proposal by Ling (*5*) that intracellular water (endosolvent) exists as multiple polarized layers adsorbed onto cell proteins.

Two lines of evidence suggested that proton signals from the water in cancerous tissue would be distinct from the radio-frequency emissions of normal tissue. My own experiments with *Escherichia coli* (*6*) suggested that altered selectivity coefficients of alkali cations in biologic tissue, such as occur in neoplastic tissue (*5*), can indicate alterations in tissue water structure. In addition, Hazlewood and his co-workers have recently reported evidence from NMR measurements that growth and maturation of skeletal muscle in the newborn rat is accompanied by simultaneous changes in water structure and

Table 1. Spin-lattice (T_1) and spin-spin (T_2) relaxation times (in seconds) of normal tissues.

Rat No.	Weight (g)	Rectus muscle		Liver		Stomach T_1	Small intestine T_1	Kidney T_1	Brain T_1
		T_1	T_2	T_1	T_2				
1	156	0.493	0.050	0.286	0.050	0.272	0.280	0.444	0.573
2	150	.548	.050	.322	.060	.214	.225	.503	.573
3	495	.541	.050	.241	.050	.260	.316	.423	.596
4	233	.576 (0.600)*	.070	.306 (0.287)*	.048	.247 (0.159)*	.316 (0.280)*	.541 (0.530)*	.620 (0.614)*
5	255	.531		.300		.360	.150	.489	.612
					Mean and standard error				
		0.538 ± 0.015	0.055 ± 0.005	0.293 ± 0.010	0.052 ± 0.003	0.270 ± 0.016	0.257 ± 0.030	0.480 ± 0.026	0.595 ± 0.007

* Spin-lattice relaxation time after the specimen stood overnight at room temperature.

in the alkali cation composition of the muscle (7). These results suggest that dedifferentiation and anaplasia, commonly equated with neoplasia, may be associated with profound alterations in endosolvent structure. These data (7) suggest the nature of the alteration in H_2O structure that should be observed for dedifferentiated or neoplastic tissue. Substantially narrower line widths [the result of a decreased ordering of cell water (2, 3)] were observed for immature skeletal muscle than for mature muscle. On this basis undifferentiated neoplastic tissue can be expected to manifest increased relaxation times (narrow line widths).

The experiments were performed with Sprague-Dawley rats previously infected with either Walker sarcoma (solid form) or Novikoff hepatoma. The rats ranged in weight from 150 to 500 g and were selected at random in order to exclude variations in the age of the rat as a material consideration (8). The animals were killed by cervical fracture when tumor size reached approximately 1.5 ml in volume (4 to 5 days after inoculation in the case of animals with Novikoff hepatoma and 10 days in the case of animals with Walker sarcoma). The samples were excised and packed in cellulose nitrate tubes. In all cases the NMR measurements were obtained within 5 minutes after the death of the animal.

The experimental arrangement consisted of an electromagnet (Varian) 12 inches (30.5 cm) in diameter operating at approximately 5610 gauss, a pulse spectrometer (Nuclear Magnetic Resonance Specialties Corporation PS-60 AW), and a probe of cross-coil design operating at 24 Mhz.

Two types of NMR experiments were performed. Spin-lattice relaxation time was measured by the method of Carr and Purcell (9). In this method a sequence of two pulses is used with pulse widths for the two pulses set to produce a 180° nutation followed by a 90° nutation. With this sequence, one observes a free induction decay after the second pulse whose amplitude is given by

$$M(\tau) = M_0(1 - 2e^{-\tau/T_1})$$

where M_0 is the equilibrium value of the pulse amplitude and τ is the interval between pulses. This equation implies that, if T_1 is multiplied by the natural logarithm of 2, the product is equal to the pulse interval that produces no free induction decay after the 90° pulse. In actual practice, once the two pulses were phased and pulse widths set for the proper nutation angle, an oscilloscope (Fairchild 766 H/F, 25 and 50 Mhz) was synchronized to trigger on the second pulse and the pulse interval was adjusted until the null free induction decay was obtained. The interval between the two pulses was obtained from a frequency counter (Computer Measurement Company 200CN) interfaced with the output of the PS-60 spectrometer programmer.

I measured the spin-spin relaxation time by using a 90° to 180° pulse sequence and making use of the Carr-Purcell modification (9) to obtain the echo decay envelope. This method for measuring T_2 is free of diffusion effects and field inhomogeneities (9). Since the envelope height E is given by

$$E(2n\tau) \propto e^{-2n\tau/T_2}$$

where n represents integral multiples of the pulse separation τ, T_2 was estimated from the oscilloscope trace as the time required for the envelope height to decay to $1/e$.

The spin echo resonance measurements are listed in Tables 1 and 2. The contrast between the relaxation rates of malignant Novikoff hepatoma and normal liver illustrates the degree of perturbation of endosolvent structure that can accompany malignant transformation. The considerable increase in relaxation times for the hepatoma (T_1, 0.826 second; T_2, 0.118 second) relative to normal liver (T_1, 0.293 second; T_2, 0.050 second) suggests a significant decrease in the degree of ordering of intracellular water (2) in malignant tissue. In addition, it is apparent from the prolonged relaxation times of the two malignant tumors reported in Table 2 that NMR techniques would make it possible for one to detect the presence of metastatic infiltrates of the liver from either Walker sarcoma or Novikoff hepatoma.

It was also found that the differences between the relaxation rates of malignant tumors and normal liver could be used to distinguish the two malignancies from all of the normal tissues studied [P values less than .01 (Table 2)]. The values of T_1 in Walker sarcoma (0.736 second) and Novikoff hepatoma (0.826 second) were significantly greater than the values of T_1 in any of the normal tissues (0.293 to 0.595 second). The

Table 2. Spin-lattice (T_1) and spin-spin (T_2) relaxation times (in seconds) in tumors.

Rat No.	Weight (g)	T_1	T_2
		Walker sarcoma	
6	156	0.700	0.100
7	150	.750	.100
8	495	.794 (0.794)*	.100
9	233	.688	
10	255	.750	
Mean and S.E.		0.736 ± 0.022	.100
P		< .01†	
		Novikoff hepatoma	
11	155	0.798	0.120
12	160	.852	.120
13	231	.827	.115
Mean and S.E.		0.826 ± 0.013	0.118 ±
P		< .01†	0.002
		Fibroadenoma (benign)	
14		0.448	
15		.537	
Mean		.492	
		Distilled water	
		2.691	
		2.690	
		2.640	
Mean and S.E.		2.677 ± 0.021	

* Spin-lattice relaxation time after the specimen stood overnight at room temperature. † The P values are the probability estimates of the significance of the difference in the means of T_1 for the malignant tumor and for brain.

values of T_2 in the malignant tumors (0.100 and 0.118 second) were about twice the values of T_2 in rectus muscle (0.055 second) and liver (0.052 second). Furthermore, replicate measurements of T_1 in the malignant tissues were found to be highly reproducible (standard error of the mean, $< .02$) and the normal tissues had a standard error of the mean of .03 or less despite deliberate scrambling of the ages and weights of the animals in the experimental colony.

On the whole, these results support the findings of Hazlewood and his co-workers (7) and are in general agreement with Szent-Györgyi's assertion that cancerous tissue has a lower degree of organization and less water structure than normal tissue (10). Furthermore, the data conformed to the results expected on the basis of a knowledge of the cation content of cancerous tissue. Dunham et al. have reported that with "few exceptions the potassium content of malignant neoplasms is increased" by comparison with that of normal cells (11). Ling has pointed out that the variations in alkali cation selectivity observed by Dunham et al. are readily explained by the association-induction hypothesis (5, p. 523). Nuclear magnetic resonance line width measurements in my laboratory (6) have demonstrated a correlation between narrowing of the line width of the cell water signal and potassium enrichment in bacteria (E. coli), which, in turn, is consistent with the aqueous properties of potassium as a "structure-breaking" agent (12). "Structure-breaking" by the alkali cations below Na in the periodic table (K, Rb, Cs), producing decreased ordering of the molecules in bulk water, results in narrowing of the NMR line width (6).

The measurements were also unaffected by any change in the elevation of the sample position in the probe, packing and repacking of the specimen, or the stepwise rotation of the sample tube in the probe through 360°. In fact T_1 proved to be even relatively unchanged after the specimens stood overnight at room temperature (Tables 1 and 2, parenthetical values for rats 4 and 8).

These studies indicate that NMR methods may be used to discriminate between two malignant tumors and a representative series of normal tissues. The results suggest that this technique may prove useful in the detection of malignant tumors.

The possibility that NMR might be used for rapid discrimination between benign and malignant surgical specimens was also considered. Relaxation times for two benign tumors (fibroadenomas) were distinct from those of the malignant tissues and were the same as those of muscle (Tables 1 and 2).

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