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Water content and water structure in CT and MR signal changes: possible influence in detection of early stroke.

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Water Content and Water Structure in CT and MR Signal Changes: Possible Influence in Detection of Early Stroke

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Recent work by the authors and others has shown that MR imaging is more sensitive than CT in the detection of acute stroke. To separate the effects of water content and water structure on MR signal intensity, we undertook two sets of experiments that used simple model systems: gelatin gels with increasing water content and hardened hens' eggs. CT and MR were performed on both systems. On CT there was a direct linear relationship between CT attenuation (Hounsfield units) and the specific gravity of the gelatin gels, and an inverse relationship with water content. There was only a minimal change in the specific gravity of egg samples with hardening and, as expected on CT, no change in linear attenuation accompanying hardening. On MR there was a linear relationship between water content in gelatin gels and spin-lattice (T1) relaxation time (r = .92, $p < .01$) and spin-spin (T2) relaxation time (r = .91, $p < .05$). However, these changes were insufficient to explain the changes of signal intensity that occur in the brain with infarction. The simple cellular system with hens' eggs demonstrated that shortening of T1 and T2 accompanied egg hardening with minimal change in water content; the shift of water from bulk water to a bound or structured form was probably the basis of this phenomenon.

We found that water structure and not merely water content is a significant mechanism underlying relaxation time changes and signal intensity changes in acute stroke.

Acute cerebral infarction produces an increase in water content of approximately 6% in the infarcted cerebral tissue [1, 2]. CT has previously been the imaging technique used most often to evaluate stroke patients. A number of clinical studies of cerebral infarction have demonstrated a greater sensitivity of MR than CT for detecting acute stroke [2–8]. While previous investigators have described the increase in water content accompanying cerebral infarction and attributed to it the high signal intensity of cerebral infarcts seen on T2-weighted spin-echo images, the effect of brain softening on MR signal intensity and T1 and T2 relaxation has not been addressed.

To understand the factors responsible for the signal intensity characteristics observed on MR and CT during stroke we studied two model systems relating water content to signal intensity and measured relaxation times as well.

Materials and Methods

Model Preparation

A series of six gelatin gels* were prepared by mixing gelatin with deionized water. Hens' eggs were placed in boiling water for 1, 2, 4, and 30 min and were cooled immediately afterward by immersing each in 1000 ml of 4°C water. Eggs varying from raw to hard-boiled were then tested for water content and specific gravity and imaged by MR and CT; measurements of T1 and T2 values were made with the techniques described below.

* Fisher Chemical Co.

Measurement of Water Content

Water content was verified by wet-to-dry measurements: approximately 100-mg gelatin or egg samples were weighed, dried in a standard oven at 100°C to constant weight (about 24 hr), and cooled in a desiccator and then reweighed. Values were reported as percentage water in the sample.

Measurement of Specific Gravity

The specific gravity of the gels and eggs was measured by modifying a density gradient technique described elsewhere [1]. Bromobenzine (specific gravity, 1.5219) and N-decane (specific gravity, 0.7300) were mixed in volume ratios of 66–87 (lower phase) and 35–65 (upper phase). Small squares of gel and egg of approximately 1 mm³ in volume were placed in the gradient. By comparing the equilibrium position of the sample in the column with that of a standard, the specific gravity was determined.

T1 and T2 Measurements

Measurements were made by using an IBM Minispec PC/20,[†] which operates at a frequency of 20 MHz and uses a permanent magnet. Specimens were adjusted for sample geometry, and appropriate phase corrections were made by using a Textronix Oscilloscope.[‡] T1 measurements were obtained by using an inversion-recovery pulse sequence (180° tau 90°) and a nonlinear least-squares fit to the curve derived from eight data points. T2 was measured by the Carr-Purcell-Meiboom-Gill technique by using 10 even-echo intensities in a nonlinear fit of the curve with two parameters [9]. The reported T1 and T2 values were averages of two separate and consecutive measurements. Accuracy of T1 measurements was tested by comparing them with standard solutions of paramagnetic ions. The results obtained were accurate when compared with reported values [10].

MR Imaging

The samples (gels and eggs) were scanned with a Siemens Magnetom operating at 0.5 T with the head coil. Data were processed for a field of view of 23 cm on a 256 × 256 matrix. A multislice, multiecho technique was used with spin-echo pulse parameters of 500/35/4 (TR/TE/acquisitions) and 1500/35, 120/2. This technique results in three images at the same sample level: a T1-weighted, a relatively proton-density-weighted, and a T2-weighted, respectively. Image planes were oriented along the long axis of the eggs and the transverse plane of the tubes containing the gels. Section thickness was 1 cm. The signal intensities of the samples on MR (gels and eggs, both white and yolk) were measured with the cursor on the cathode ray tube monitor by selecting a region of interest.

CT Scanning

CT imaging was performed on a Siemens DRH scanner at 125 kVp and 45 mAs processed for a field of view of 25.6 cm on a 512 × 512 matrix. The eggs were scanned along their long axes, the tubes of gelatin transversely. Contiguous scans were obtained with 8-mm collimation. All data from MR and CT imaging were stored on magnetic media, and transparencies were made as well.

[†] IBM Bruker Spectrometer.
[‡] Model 565.

Results

Water Content and Specific Gravity

Water content of the gelatin gels varied from 76 to 91% (Table 1). The corresponding changes in specific gravity varied from 1.0875 to 1.0322. Statistical analysis of the relationship between water content and specific gravity of the gels showed a linear relationship, $r = .998$ ($p < 10^{-5}$). The regression analysis yielded a specific gravity of $1.0057 + 0.0036 \times$ water content. Analysis of the water content of egg white and yolk after hardening showed only minimal change in water content of 1.2% between raw and 4-min egg white and minimal, if any, change in specific gravity (Table 2).

Relaxation Values

Gelatin relaxation values calculated by spectrometry are listed in Table 1. For the gels, T1 ranged from 0.914 to 2.118 sec and T2 from 0.419 to 1.215 sec. The relationship between water content of the gelatin gels and T1 and T2 was directly linear. The linear regression for water content and T1 was $y = -5.869 + 0.0866x$, $r = .92$ ($p < .01$). Linear regression analysis of the relationship between gel water content and T2 showed $y = -3.996 + 0.0561x$, $r = .913$ ($p < .05$). Figure 1 shows a plot of T1 and T2 vs water content of the gelatin gels. Egg hardening, with only 1 min of boiling, resulted in profound T2 shortening and lesser shortening of T1. Changes observed beyond the initial minute of boiling were less marked (Table 2).

MR Imaging

Signal intensities of the gels with varying water contents are listed in Table 1. There was a gradual increase in signal intensity of approximately 50%, with maximal signal intensity at 87% water content on the T2-weighted image. Figure 2 shows the MR images of the eggs obtained on the various pulse sequences. The signal intensity of the raw and hardened eggs is listed in Table 2. There was a dramatic decrease in signal intensity on the T2-weighted images accompanying a small degree of egg hardening. The findings on T1 and proton-density imaging were less striking; there was little change in signal intensity of egg white accompanying hardening. The signal intensity of yolk did not vary much with the changes of hardening on any pulse sequence.

CT Scanning

CT attenuation values of the gelatin gels are listed in Table 1. Linear regression analysis of the relationship between water content and CT attenuation was $y = 287.5 - 2.622x$, $r = .983$ ($p < .0005$). Statistical analysis of the relationship between CT attenuation and specific gravity showed a correlation coefficient for linearity of 0.992 ($p < 10^{-4}$). Regression analysis yielded a CT density (H) of $-692.607 + 717.195 \times$ specific gravity. In the eggs there was no appreciable change on CT attenuation accompanying hardening (Table 2).

TABLE 1: Changes in CT Density, MR Signal Intensity, and Relaxation Times with Water Content of Gels

% Water	Specific Gravity	CT Density (H)	MR Signal Intensity			MR Relaxation Time (sec)	
			500/35	1500/35	1500/120	T1	T2
91	1.0322	46.0 ± 0.6	475 ± 29	1643 ± 106	1278 ± 149	2.118 ± 0.022	1.215 ± 0.003
87	1.0482	59.9 ± 1.1	679 ± 35	1809 ± 148	1336 ± 116	1.844 ± 0.033	0.973 ± 0.004
86	1.0538	63.7 ± 0.1	750 ± 34	1845 ± 139	1306 ± 91	1.447 ± 0.028	0.696 ± 0.002
83	1.0636	70.5 ± 1.2	763 ± 28	1716 ± 115	1141 ± 73	1.061 ± 0.027	0.552 ± 0.003
81	1.0716	78.4 ± 1.3	805 ± 46	1748 ± 148	1120 ± 80	1.035 ± 0.040	0.429 ± 0.008
76	1.0875	85.0 ± 1.4	694 ± 40	1423 ± 109	837 ± 51	0.914 ± 0.033	0.419 ± 0.003

TABLE 2: Changes in CT Density, MR Signal Intensity and Relaxation Times with Egg Hardening

| Time Boiled	% Water	Specific Gravity	CT Density (H)	MR Signal Intensity			MR Relaxation Time (sec)	
				500/35	1500/35	1500/120	T1	T2
White (n = 5):								
Raw	87.98	1.0482	54.7 ± 2.9	646 ± 2	1286 ± 46	1238 ± 74	1.319 ± 0.011	0.560 ± 0.001
1 min	86.58	1.0276	51.5 ± 6.8	457 ± 32	1313 ± 1.4	417 ± 110	1.145 ± 0.018	0.136 ± 0.002
2 min	86.06	1.0325	51.8 ± 1.0	508 ± 30	1254 ± 23	248 ± 74	0.976 ± 0.015	0.082 ± 0
4 min	86.76	1.0300	51.5 ± 6.8	529 ± 29	1551 ± 227	218 ± 4	0.934 ± 0.011	0.069 ± 0
30 min	84.99	1.0446	54.7 ± 2.9	581 ± 24	1462 ± 62	163 ± 1	0.892 ± 0.015	0.067 ± 0
Yolk (n = 5):								
Raw	52.07[a]	1.0399	20.3 ± 3.1	446 ± 33	286 ± 46	33 ± 16	0.069 ± 0.022	0.034 ± 0.001
1 min	53.05	1.0367	19.2 ± 1.6	259 ± 16	313 ± 1	42 ± 4	0.065 ± 0.004	0.038 ± 0.001
2 min	52.99	1.0338	16.6 ± 3.8	306 ± 19	254 ± 23	22 ± 11	0.065 ± 0.004	0.027 ± 0.001
4 min	55.55	1.0308	24.3 ± 2.6	339 ± 52	551 ± 227	67 ± 37	0.066 ± 0.004	0.026 ± 0.001
30 min	54.93	1.0413	29.4 ± 2.6	341 ± 30	462 ± 62	52 ± 3	0.066 ± 0.001	0.028 ± 0.001

[a] SD = 2.93%.
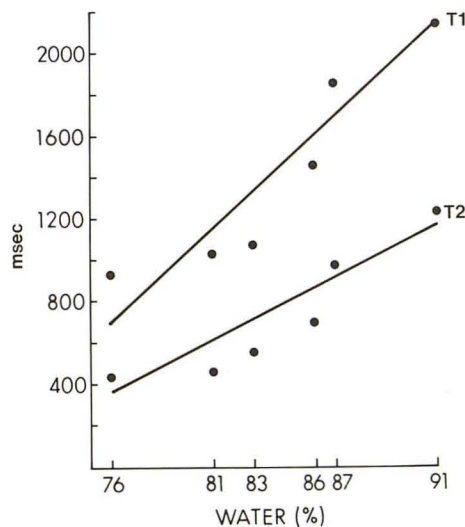
Fig. 1.—Changes of T1 and T2 with changes of water content in gelatin gels.

Discussion

The changes that occur in stroke and its subsequent cerebral edema are complex. Not only are there changes in water content and changes in the concentration of intracellular and extracellular solutes [11], but, in addition, there is disruption of the subcellular compartments and denaturation of proteins and lipoprotein membranes [12]. MR images reflect all of these processes.

The processes responsible for the changes in linear attenuation that are observed on CT with stroke or brain edema are simpler than those in MR. With increased water content there is decreased specific gravity of brain tissue. CT attenuation is linearly proportional to specific gravity [13], hence the CT changes in the gel model and the lack of such changes in the egg hardening model. The data from CT attenuation vs changes in water content in the current experiment show an approximate change of 2.6 H for every 1% change in water content in the gels. This compares well with the work of Mano et al. [4], who used a similar range of water concentration in polyvinyl alcohol bars and found a change of 2.5 H per percentage change in water content and a similar linear proportionality.

Comparative studies of CT and MR have generally shown that MR is more sensitive than CT in the detection of acute ischemic stroke (within the first 24 hr) [4–8]. In our previous work in primates MR was positive 2–4 hr after stroke, and we observed an approximate 90% increase in T2 signal intensity of infarcted cerebral tissue accompanying a 6% increase in water content [2].

The changes in signal intensity observed on MR are complex and are described by the signal equations. For the commonly used spin-echo sequence, signal intensity is related to four variables: proton (spin) density, T1 and T2 relaxation times, and flow [14]. The increased water content of brain edema results in prolongation of both T1 and T2 [15, 16]. A number of experimental studies have shown that proton MR demonstrates cortical changes before 24 hr [8, 17–20]. Such studies have generally shown decreased signal intensity on
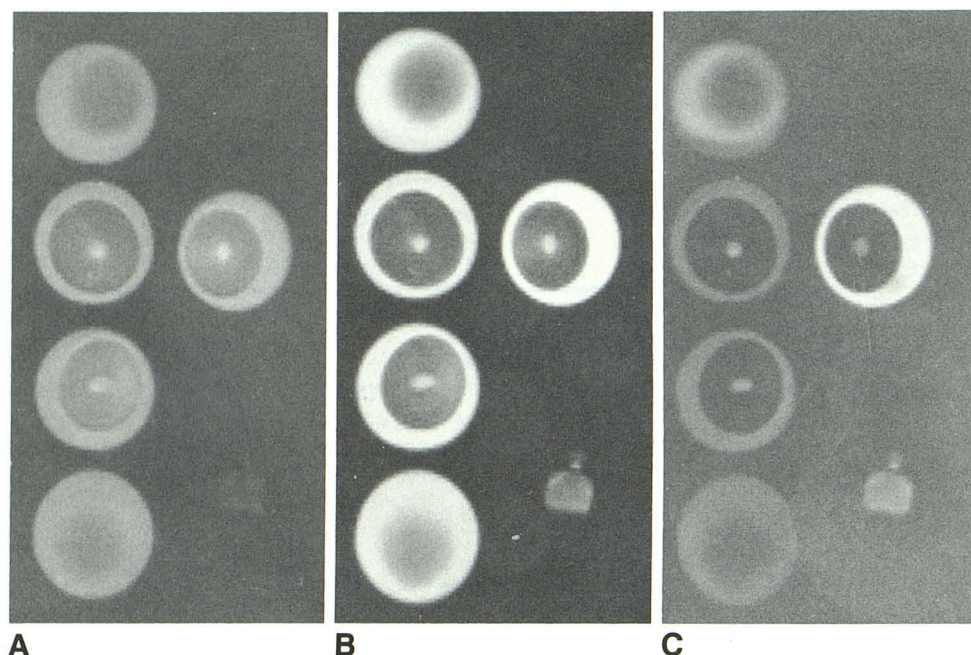
T1-weighted images and increased signal on T2-weighted images [2, 16, 17]. Some experimental and clinical studies derived calculated T1 images or T2 images [15] and documented prolongation of T1 and T2 relaxation times within 24 hr [2, 21–23]. Studies measuring T1 and T2 relaxation times of tissue samples likewise showed progressively increasing relaxation times with increasing water content [15, 17, 24, 25]. Some authors have hypothesized that a prolongation in T1 and T2 relaxation times and increasing interhemispheric difference in signal intensity after unilateral carotid artery occlusion probably reflect focal edema that developed as a result of ischemic cerebral lesions [21]. However, other authors have suggested that the changes in relaxation times with brain edema may not be due to changes in percentage water content alone but also to changes in the structure of water [6, 24]. Ngo et al. [26] showed that two analogues of adriamycin produced similar effects of cellular progression arrest and increased cellular water increments in cell culture. However, adriamycin produced prolongation of T1 and T2 while the analogue AD-37 produced no change in T1 and only a 10% increase in T2, illustrating that a mere change in total water content was not alone sufficient to explain changes in T1 and T2 relaxation.

In this study we have attempted to investigate separately the changes of water content and water structure. Our experiments demonstrated that in a gelatin gel model there was a linear relationship between water content, T1, and T2. Inch et al. [27] demonstrated a similar linear relationship between T1 and water content of gelatin over a similar range of water contents. However, an increase in water content of 6% in the gels resulted in only a 19% increase in signal intensity of gel on the T2-weighted image (Table 1). This is in comparison with a 25% change in CT attenuation in the same specimens. This is a paradox in light of the higher sensitivity of MR than CT reported by us and other investigators over a similar range

of water contents in stroke. In our previous work on stroke in a primate model we found that a 6% increase in water content resulted in a 90% increase in signal intensity on the T2-weighted image. Certainly the gels are a simple system to compare with water content changes in the brain, but the gelatin experiments illustrate how mere changes in water content are probably insufficient to explain changes in signal intensity accompanying infarction. Brain "softening" likely is an additional variable in stroke that affects signal intensity. This is further suggested in our egg experiment by profound changes in the signal of egg whites with hardening, accompanied by only minimal change in water content or specific gravity. Others have described several fractions of water in protein solutions, cells, and biological systems [28–31]. Over the range of water contents in nonischemic mammalian cells, much of the water is involved in a structured or bound form in close relationship with protein molecules. The small fraction of bulk or free water that is present in cells will have the longest T1 and T2 relaxation values. The mean T1 and T2 calculated for a tissue will reflect the mean relaxation values for the different components of water in the various fractions [30]. Accompanying egg hardening are a dramatic T2 shortening and a modest T1 shortening. This most likely reflects a decrease in the bulk water in the egg white and a transfer of this water into either the bound or structured water compartments, which results in shortening of T1 and T2.

Our data from MR of gels show an increase in signal intensity on the T2-weighted image of only about 60%, with an 11% increase in water content. This is much lower than the observed increase in signal intensity of cerebral tissue with a correspondingly much smaller increase in water content. Previous work has shown that gelatin will bind water to approximately 55% by weight in the form of a bound and structured water compartment. The range of gelatin water contents evaluated in this study (76–91%) corresponds to

increases in bulk water well above the threshold of the bulk water fraction. In the case of the brain nearly all of the intracellular water is probably bound or structured in the nonischemic cerebral tissue. A small increase in water content likely causes a disproportionate increase in the bulk water fraction, causing prolongation of T1 and T2. A subtle change in water content in the bulk water fraction might be detectable on MR, whereas a subtle change in water content might not be detected by CT. Brain softening likely reflects this increase in water in the bulk fraction and provides an adequate explanation of the magnitude of signal-intensity changes observed in cerebral infarction with MR.

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