Low Sensitivity of Clinical MR Imaging to Small Changes in the Concentration of Nonparamagnetic Protein

David B. Hackney[1]
Robert I. Grossman
Robert A. Zimmerman
Peter M. Joseph
Herbert I. Goldberg
Larissa T. Bilaniuk
Marie V. Spagnoli[2]

This study attempts to determine the magnitude of change in the concentration of a nonparamagnetic protein (human serum albumin) required to effect a detectable change in signal intensity on a clinical imaging unit. For a range of protein concentrations from 0–6100 mg/dl the concentration could not be predicted by inspecting the images. Measurement of displayed signal intensity failed to distinguish concentrations of 0.09–3700 mg/dl, while 6100 mg/dl gave slightly higher intensity signals. Although this low sensitivity represents expected behavior for low concentrations, the failure to differentiate the higher concentrations implies limitations imposed by clinical imaging techniques.

Our results suggest that additional factors, such as paramagnetic material and motion as well as differences in protein concentration, may be involved in the MR signal intensities observed in pathologic CSF and cystic CNS collections.

Cystic lesions of the CNS are now being evaluated with MR imaging at every opportunity. Fluid-containing structures demonstrate prolongation of T1 and T2 when compared with brain or spinal cord parenchyma [1, 2]. The signal characteristics of fluid within these cystic collections suggest a shorter T1 than CSF (Fig. 1). There often is a higher protein concentration in these collections than in normal CSF. This has led to the postulate that the signal-intensity variations are a result of the higher protein concentrations of the cyst fluid than are found in CSF [1, 3]. The influence of dissolved protein on solvent proton MR relaxation rates has been thoroughly characterized for aqueous solutions [4–18]. As the protein concentration increases there is an increase in the rapidly relaxing water fraction. This will shorten both T1 and T2. However, the concentrations of protein required to effect significant changes in relaxation rates have not been correlated with the range of clinically relevant protein concentrations. This lack of documentation has led to speculation that small changes in protein concentration, on the order of tens to hundreds of mg/dl, may cause profound changes in signal intensity on clinical images [1, 3]. Since this suggests far greater sensitivity to small changes in concentration than have been observed with laboratory data [4–18] we constructed a study to address the question: Can experienced observers reliably recognize and rank small differences in protein concentration by inspecting hard-copy images generated with routine clinical pulse sequences?

Received May 14, 1986; accepted after revision June 21, 1987.

Presented at the annual meeting of the American Society of Neuroradiology, San Diego, January 1986.

[1] All authors: Department of Radiology, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104. Address reprint requests to D. B. Hackney.

[2] Present address: Department of Radiology, Saint Luke's Memorial Hospital Center, P.O. Box 479, Utica, NY 13503.

AJNR 8:1003–1008, November/December 1987
0195–6108/87/0806–1003

Materials and Methods

Protein Solutions

Normal saline and human serum albumin were combined to prepare a series of protein concentrations ranging from 0–6100 mg/dl. Protein concentrations were determined by clinical laboratory methods.

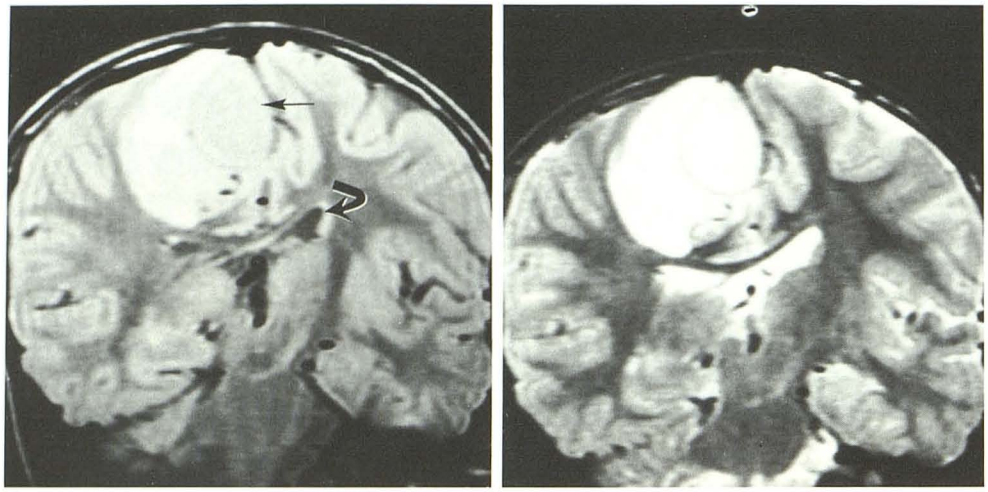

Fig. 1.—Coronal spin-echo images of parasagittal subdural empyema.

***A*, Early-echo image, TR = 2500 msec, TE = 30 msec. Collection (*straight arrow*) has higher signal intensity than ventricular fluid (*curved arrow*), suggesting shorter T1 of the former.**

***B*, Later-echo image, TR = 2500 msec, TE = 80 msec. Both collection and CSF appear much brighter than brain, but there is very little contrast between CSF and empyema.**

Phantoms

The protein phantom consisted of 12 plastic tubes, each filled with one of the solutions and arranged in random order with respect to protein concentration (Fig. 2). The diameter of each tube was 2 cm. A uniformity phantom consisted of a basketball filled with a copper sulfate solution (Fig. 3).

Phantom Scanning

The phantoms were scanned on a clinical 1.5-T GE Signa MR unit. The pulse sequences had a repetition time (TR) = 600 msec, echo time (TE) = 25 and 50 msec with four signal averages or TR = 2500 msec, TE = 25, 50, 75, and 100 msec with two averages. The field of view was 32 cm and the matrix 128 × 256. This yielded a nominal pixel size of 2.5 × 1.25 mm. Thus, the cross-sectional area of each tube corresponded to approximately 100 pixels.

Image Signal Intensity Determinations

Objective.—By using the region-of-interest signal-intensity function, the intensity of each tube was measured for each pulse sequence. A square region of interest was used with the largest area that would fit completely within the circular cross section of the tube. A background value was derived by averaging the intensity of air at seven points around and between the tubes.

Subjective.—The images resulting from each pulse sequence were photographed at a variety of window and level settings. The images were reviewed independently by five neuroradiologists experienced in MR without knowledge of the protein concentration. Each reviewer ranked the intensity of each tube for each set of pulse-sequence parameters from 1 (lowest intensity) to 12 (highest). The rankings were averaged for each cylinder, and the results for the TR = 600, TE = 25 and TR = 2500, TE = 100 parameters are presented in Table 1.

Results

The images derived from the TR = 600 msec, TE = 25 msec and TR = 2500 msec, TE = 100 msec pulse sequences are displayed in Figure 2. Although the protein concentrations ranged from 0.09–6100 mg/dl there was little consistent change in signal intensity. Cylinder E had the highest protein concentration and appeared slightly more intense than the other solutions on images derived from all pulse-sequence parameters. Cylinder E was the only solution for which the signal intensity clearly correlated with the protein concentration.

An image of the copper sulfate phantom is reproduced in Figure 3. When wide windows were used (as for clinical images) the image appeared reasonably uniform. However, when the windows were narrowed drastically, as was necessary to detect the small differences in signal intensity among the protein cylinders, the nonuniformity of the field became apparent. Most importantly, we noticed that the relative signal intensities of the protein cylinders in Figure 2 paralleled the relative signal intensities at various positions in the head coil in Figure 3. This indicates that the intensity differences in Figure 2 were largely caused by the inhomogeneous response of the coil rather than by intrinsic differences in the relaxation behavior of the protein solutions.

Figure 4 plots the measured net signal intensity (signal − background) against protein concentration for the shortest and longest spin-echo sequences. The pattern is similar to Figure 2, demonstrating wide scatter at the lower protein concentrations. The highest-concentration solution (cylinder E) has a consistently higher signal intensity than the other solutions. Even at this high concentration the higher signal intensity may to some extent represent artifact. Cylinder E was positioned in a high-intensity region of the coil (Figs. 2 and 3). The extremely flat shape of the curves in Figure 4 suggests that there is very little sensitivity to changes in protein concentration of up to 3700 mg/dl and perhaps higher. This implies that there are only very small changes in signal intensity between the different solutions and that these changes are easily obscured by noise and by the inhomogeneous response of the clinical unit at various positions in the field.

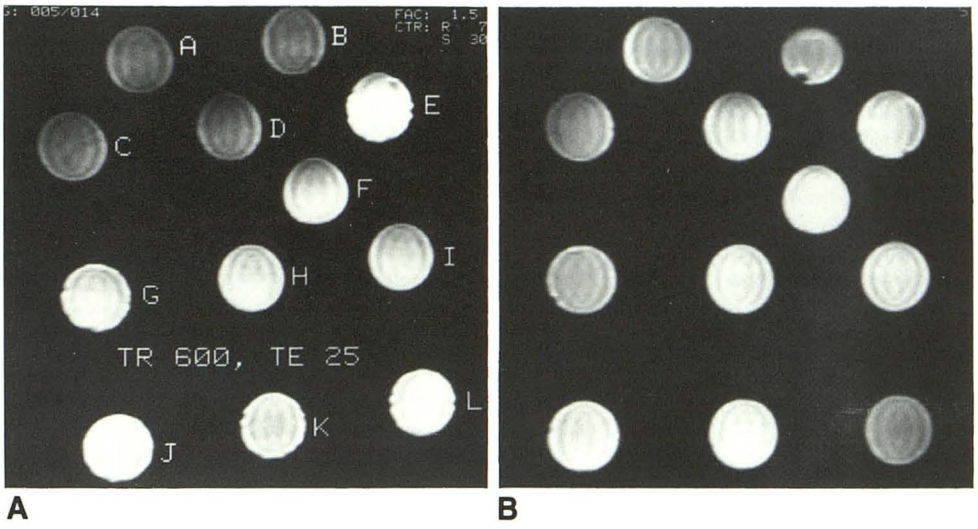

Fig. 2.—Short (TR = 600 msec, TE = 25 msec, *A*) and long (TR = 2500 msec, TE = 100 msec, *B*) spin-echo images of phantom in same orientations. Significant inhomogeneity is apparent. While this appears particularly striking on phantom images, it is present on clinical images but does not preclude obtaining high-quality images (Fig. 1).

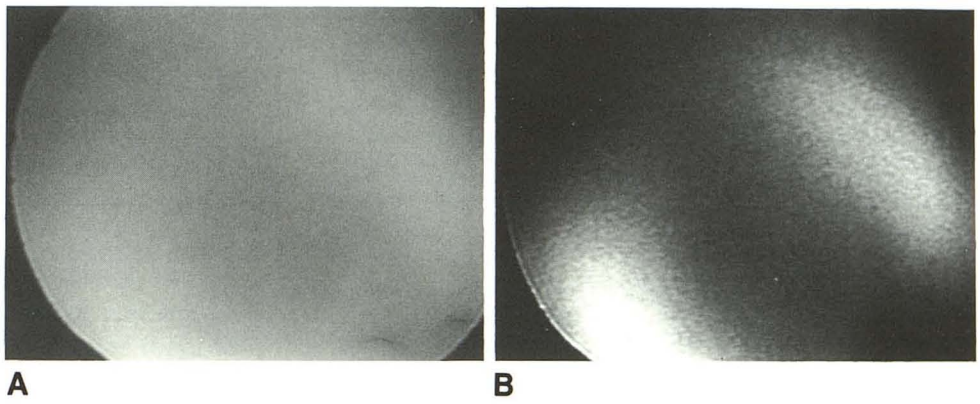

Fig. 3.—Wide (*A*) and narrow (*B*) window displays of copper-sulfate-uniformity phantom image with TR = 600 msec, TE = 25 msec. Note wide variations in signal intensity on narrow window image from areas with identical fluid compositions.

TABLE 1: Subjective Relative Signal Intensity of Protein Solutions

Cylinder	Protein Concentration (mg/dl)	Relative Signal Intensity	
		TR = 600 msec, TE = 25 msec	TR = 2500 msec, TE = 100 msec
A	0.09	1	4.6
I	0.09	5.8	5.8
B	10	2.2	2.2
H	11	8	8.8
L	45	10.6	1
G	100	8	3.8
D	108	3.2	7.2
F	278	7	10.4
C	480	2	2
K	1040	5	10.2
J	3700	9	7.8
E	6100	11.6	7.4

Note.—Relative signal intensity is the average of five independent rankings on a 1–12 scale, where 1 = lowest intensity and 12 = highest.

Discussion

Elevated protein content in CSF is a nonspecific finding associated with a variety of pathologic processes. Pathologic fluid collections in the CNS tend to have higher protein concentrations than normal CSF. Because adding protein to water reduces T1 and T2 [4–18], we might expect to see detectable changes in signal intensity. To explain our failure to observe such changes over a wide range of protein concentrations we must consider the absolute sensitivity of MR to protein-concentration differences and limitations in this sensitivity imposed by clinical imaging. To relate the sensitivity of MR to protein concentration on the basis of signal intensities observed in clinical imaging we must consider the levels of protein observed in CSF and pathologic fluid collections in the CNS. In reports of CSF analysis related to a variety of pathologic processes, protein rarely exceeded 2000–3000 mg/dl [19–27]. In the great majority of cases protein concentrations were much lower. Fluid-containing CNS lesions may have protein concentrations of several thousand mg/dl [19–27]. However, concentrations comparable to normal CSF or elevations of several hundred mg/dl are far more common.

The effects of soluble proteins on the relaxation rates of water protons have been studied in detail [4–18]. The water proton relaxation rate in a dilute protein solution may be expressed by $1/T_i = 1/T_{if} + k_i c$, where T_i = T1 or T2 of the protein solution $T_{if} = T_i$ of pure water, k_i = a constant (cm^3/

sec · g) empirically determined for each protein, and c = protein concentration (g protein/cm^3 solution) [14]. By using this relationship it is possible to calculate expected T1 and T2 values as a function of protein concentration. We performed these calculations for albumin by using data reported by a number of authors [10, 14, 28–30]. This indicates that adding 100 mg/dl of albumin to pure water would reduce T1 by approximately 1.5% and T2 by approximately 4%. These small changes are unlikely to be reflected as detectable signal-intensity changes. Similar reasoning reported by Hopkins et al. [30] has suggested that the low protein concentration normally present in CSF does not result in T1 or T2 values measurably different from those of pure water. Although protein concentrations on the order of several thousand mg/dl would be expected to cause significant changes in relaxation rates, a combined imaging and spectroscopy study failed to demonstrate reliable T1 changes over an albumin concentration range of 0–8000 mg/dl with a 1.5-T imaging unit [31]. The authors speculated that limitations imposed by imaging, such as gradient and radiofrequency pulse imperfections as well as inappropriately short maximal TR and TE values, may have been responsible for the poor performance of MR imaging in detecting these T1 differences.

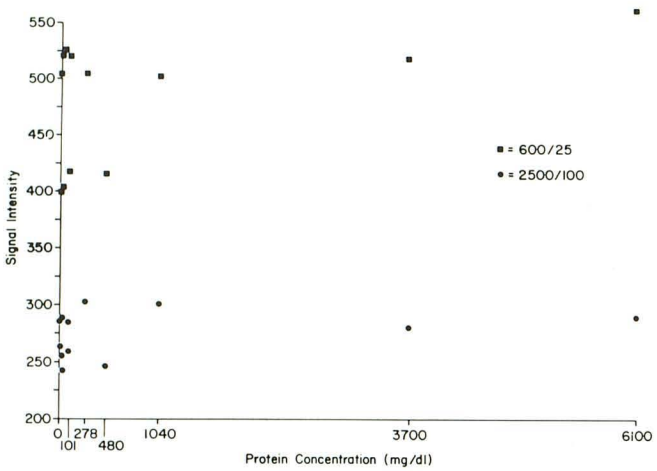

Fig. 4.—Measured signal intensity (signal − background) vs protein concentration for sequences with TR = 600 msec, TE = 25 msec and TR = 2500 msec, TE = 100 msec. Note scatter of values and absence of trend as protein concentration ranges over five orders of magnitude.

Protein in solution has a greater effect on spin-spin than on spin-lattice relaxation rates [7, 10, 11]. However, as Kjos et al. [3] have noted, with the values of TR and TE commonly used for clinical imaging, the contrast between CSF and fluid-containing CNS lesions is determined largely by differences in T1. In a spin-echo sequence the relative rates of decay of signal from CSF and cystic lesions on successive echoes reflect differences in T2. When evaluating cystic CNS lesions we perform a spin-echo pulse sequence with a long TR (3500

TABLE 2: Reported Relaxation Rates of Protein Solutions

Protein [Ref. No.]	Concentration (mg/dl)	T1 (sec)	T2 (sec)	Frequency (MHz)	Field (T)
Ovalbumin [11]	6500	1.3	0.6	20	0.47
Ribonuclease [11]	9100	1.4	0.6	20	0.47
Bovine serum albumin [11]	9100	1.1	0.6	20	0.47
Blood [28]	NA[a]	1.04	0.265	20	0.47
Plasma [28]	NA	1.44	0.49	20	0.47
Albumin/globulin solution [28]	4500/2700	1.51	0.599	20	0.47
Bovine serum albumin [8]	2000	1.7		24.3	0.57
	5000	1.2		24.3	0.57
Hemocyanin [32]	5000	0.29		0.1	0.002
		0.49		1	0.02
		1.09		10	0.2
Gamma globulin [32]	5000	0.55		0.1	0.002
		0.91		1	0.02
		1.69		10	0.2
Alcohol dehydrogenase [32]	5000	0.59		0.1	0.002
		0.81		1	0.02
		1.51		10	0.2
Carbomonoxyhemoglobin [32]	5000	0.96		0.1	0.002
		1.07		1	0.02
		1.74		10	0.2
Carbonic anhydrase [32]	5000	1.2		0.1	0.002
		1.26		1	0.02
		1.79		10	0.2
Lysozyme [32]	5000	1.56		0.1	0.002
		1.69		1	0.02
		1.9		10	0.2
Hemoglobin in sickle cell anemia [33]	30,000–32,000	0.26–0.29	0.021–0.040	44.4	1

[a] NA = not available.

Note. T1 and T2 values are as reported in references [8, 28, 33] or are derived from graphs presented in references [11, 32].

msec) and multiple echoes as late as 160 msec in order to detect differences in signal intensity related to different rates of spin-spin relaxation. Currently, we cannot obtain TRs or TEs longer than these. Ideally, the TR would be several times longer than the T1 of the most slowly relaxing component. For dilute solutions such as CSF this would require TRs on the order of 12–20 sec and would result in prohibitively long scanning times.

Table 2 lists T1 and T2 values for a number of proteins at various concentrations and field strengths. Since the T1 and T2 of pure water are not reported in most of these studies, it is not possible to directly compare protein solution relaxation rates obtained in one source with those reported in another. Note that the concentrations used are all quite high. The small changes in relaxation rates that result from very small changes in protein concentration would be extremely difficult to measure. In one system a protein concentration of at least 1 g/dl (1000 mg/dl) was required to obtain reliable results [5]. The magnitude of relaxation-time shortening expected from the addition of 100 mg/dl to water is less than or comparable to the error in measurements obtained under carefully controlled spectroscopy conditions [4, 34, 35]. In routine clinical imaging our sensitivity for detecting small changes in relaxation rates should be considerably lower.

Although the effect of dissolved protein on spin-lattice relaxation rates is field-dependent [4–6, 12, 13, 34], this effect is most prominent at field strengths far below those used for clinical MR imaging. For protein solutions of 8000–20,000 mg/dl dramatic concentration-related changes in spin-lattice relaxation rates were observed at frequencies of 0.01–0.5 MHz (approximately 0.00023–0.012 T), with relaxation rates converging toward one another and toward that of protein-free buffer as the field strength entered the 1- to 10-MHz (0.023- to 0.23-T) range [4].

We have not succeeded in determining a precise minimum albumin-concentration change detectable on our imaging unit. This measurement is complicated by the inhomogeneous response of the head coil and the absence of internal reference standards in the phantom. In clinical cases there are internal standards such as brain and CSF adjacent to the cystic lesions. Therefore, it is possible to compare signal intensities of structures in similar regions of the coil, thus reducing the significance of this inhomogeneous response. In our phantom no such internal standard was available, and reviewers were forced to compare signal intensities across the entire field. We would expect this to obscure the smallest changes in intensity resulting from small changes in protein concentration. This suggests that under ideal circumstances concentration changes of less than 3700 mg/dl may be distinguishable on clinical images. However, the 6100 mg/dl solution was in a high-signal region of the coil (Figs. 2 and 3), thus augmenting its increased signal. The extraordinarily flat slopes of the curves in Figure 4 suggest that at 3700 mg/dl the T1 shortening effect may be too weak to overcome this severity of inhomogeneity. This inhomogeneity is far more apparent on phantom images (Figs. 2 and 3) than on clinical images photographed at standard windows (Fig. 1).

It is unlikely that pathologic CNS collections contain pure water and a single purified protein. It seems more probable that the fluid contains a complex mixture of proteins, other macromolecules, and compounds of varying molecular weight and paramagnetic content. The appearance of such a collection on MR images will be determined by the combined effects of all the constituents of the fluid. The observation that the liquid components of neoplasms and abscesses have more rapid spin-lattice relaxation than CSF has [1, 3] is presumably a reflection of the higher concentrations of many substances in these lesions. We have chosen human serum albumin as our test protein and we have cited water proton relaxation information reported for several other proteins in solution. The size of the molecule, as well as its concentration, is important in determining relaxation rates. Small proteins (such as albumin) tumble relatively rapidly. Therefore, they change water relaxation rates less dramatically than do larger macromolecules, which reorient more slowly [5, 32]. Molecules associated with cell membranes or macroscopic debris would even more profoundly prolong correlation times of bound water. This differential effect based on protein molecular weight is also field-dependent, is most prominent at low fields, and is far less significant at frequencies above 10 MHz (approximately 0.23 T) [5, 32].

Although it appears unlikely that differences in protein concentration on the order of tens to hundreds of mg/dl result in detectable changes in signal intensity, the proteins may be present in association with paramagnetic species, which can have dramatic effects on relaxation rates at relatively low concentrations [28, 36]. Unfortunately, the effects of paramagnetic ions in pure water do not predict their effects on relaxation rates when present in association with macromolecules [28, 36]. The nature of the binding of a paramagnetic species to protein and the access of water to the paramagnetic center are important in determining the resulting effects on relaxation rates [37]. This suggests that an understanding of the signal characteristics of cystic collections may require a thorough analysis of the paramagnetic compounds and molecular composition of the fluid.

Blood degradation products are a common in vivo source of paramagnetic compounds. The possibility of old hemorrhage should be considered when these signal-intensity variations are observed. Other paramagnetic compounds, as yet uncharacterized, may also explain some deviations from expected signal characteristics.

Motion during imaging may influence the signal intensity of structures [38]. In the case of CSF, pulsatile motion may result in signal voids, boundary-layer phase dispersion, and ghost images [39]. These artifacts can produce lower signal intensity in moving than in stationary fluids. It seems possible that in some cases the different signal characteristics of cystic CNS lesions and CSF may in part be from greater motion in the CSF.

Finally, we should recognize that the causes of many phenomena displayed on MR images remain unknown. We agree that the fluid in many cystic CNS lesions has a higher signal intensity than normal CSF and that this observation [1, 3] is often diagnostically useful and clinically significant. It seems plausible that the signal-intensity differences often reflect differences in composition between the cyst fluid and CSF. However, we do not believe that the details of the

relevant composition differences have been defined, and we consider it unlikely that intensity variations are always determined by the same factor.

REFERENCES

1. Brant-Zawadzki M, Kelly W, Kjos B, et al. Magnetic resonance imaging and characterization of normal and abnormal cerebrospinal fluid (CSF) spaces. Initial observations. *Neuroradiology* **1985**;27:3–8
2. Lee BCP, Zimmerman RD, Manning JJ, Deck MDF. MR imaging of syringomyelia and hydromyelia. *AJNR* **1985**;6:221–228
3. Kjos BO, Brant-Zawadzki M, Kucharczyk W, Kelly WM, Norman D, Newton TH. Cystic intracranial lesions: magnetic resonance imaging. *Radiology* **1985**;155:363–369
4. Koenig SH, Schillinger WE. Nuclear magnetic relaxation dispersion in protein solutions. I. Apotransferrin. *J Biol Chem* **1969**;244:3283–3289
5. Hallenga K, Koenig SH. Protein rotattional relaxation as studied by solvent ^{1}H and ^{2}H magnetic relaxation. *Biochemistry* **1976**;15:4255–4264
6. Koenig SH, Bryant RG, Hallenga K, Jacob GS. Magnetic cross relaxation among protons in protein solutions. *Biochemistry* **1978**;17:4348–4358
7. Brown JJ, vanSonnenberg E, Gerber KH, Strich G, Wittich GR, Slutsky RA. Magnetic resonance relaxation times of percutaneously obtained normal and abnormal body fluids. *Radiology* **1985**;154:727–731
8. Saryan LA, Hollis DP, Ecnonmou JS, Eggleston JC. Brief communication: nuclear magnetic resonance studies of cancer. IV. Correlation of water content with tissue relaxation times. *JNCI* **1974**;52:599–602
9. Floyd RA, Yoshida T, Leigh JS Jr. Changes of tissue water proton relaxation times during early phases of chemical carcinogenesis. *Proc Natl Acad Sci USA* **1975**;72:56–58
10. Caputa K, Hennel JW. Proton magnetic relaxation in protein solutions. Dependence on viscosity and temperature. *Colloque Ampere* **1967**; 14:128–130
11. Abetsedarskaya LA, Miftakhutdinova FG, Fedotov VD, Mal'tsev NA. Proton relaxation in some protein solutions and gels. *Mol Biol* (Mosk) **1967**;1: 381–390
12. Koenig SH, Brown RD, Adams D, Emerson D, Harrison CG. Magnetic field dependence of 1/T1 of protons in tissue. *Invest Radiol* **1984**;19:76–81
13. Koenig SH, Brown RD. The importance of the motion of water for magnetic resonance imaging. *Invest Radiol* **1985**;20:297–305
14. Daszkiewicz OK, Hennel JW, Lubas B. Proton magnetic relaxation and protein hydration. *Nature* **1963**;200:1006–1007
15. Kuntz ID, Kauzman W. Hydration of proteins and polypeptides. *Adv Protein Chem* **1974**;28:239–345
16. Fung BM, Witschell JR, McAmis LL. The state of water on hydrated collagen as studied by pulsed NMR. *Biopolymers* **1974**;13:1767–1776
17. Bryant RG, Shirley WM. Water-protein interactions. Nuclear magnetic resonance results on hydrated lysozyme. In: Rowland SP, ed. *Water in polymers*. Washington, DC: American Chemical Society, **1980**:147–155
18. Lindstrom TR, Koenig SH. Magnetic-field-dependent water proton spin-lattice relaxation rates of hemoglobin solutions and whole blood. *J Mag Reson* **1974**;15:344–353
19. Stevens JM, Olney JS, Kendall BE. Post-traumatic cystic and non-cystic myelopathy. *Neuroradiology* **1985**;27:48–56
20. Rossier AB, Foo D, Shillito J, Dyro FM. Post traumatic cervical syringomyelia. *Brain* **1985**;108:439–461
21. Barsoum A, Cannillo KL. Thoracic constrictive arachnoiditis after Pantopaque myelography: report of two cases. *Neurosurgery* **1980**;6:314–316
22. Kandt RS, Shimnnar S, D'Souza BJ, Singer HS, Wharam MD, Gupta PK. Cerebrospinal metastases in malignant childhood astrocytoma. *J Neurooncol* **1984**;2:123–128
23. Kim KS, Ho SU, Weinberg PE, Lee C. Spinal leptomenigeal infiltration by systemic cancer: myelographic features. *AJR* **1982**;139:361–365
24. Kithara M, Katakura R, Wada T, Namiki T, Suzuki J. Diffuse form of primary leptomeningeal gliomatosis. *J Neurosurg* **1985**;63:283–287
25. Barcena A, Lobato RD, Rivas JJ, et al. Spinal metastatic disease: analysis of factors determining functional prognosis and the choice of treatment. *Neurosurgery* **1984**;15:820–827
26. Fischer G, Mansuy L. Total removal of intramedullary ependymomas: Follow up study of 16 cases. *Surg Neurol* **1980**;14:243–249
27. Sloof JL, Kernohan JW, MacCarty CS. *Primary intramedullary tumors of the spinal cord and filum terminale*. Philadelphia: Saunders, **1964**
28. Kang YS, Gore JC, Armitage IM. Studies of factors affecting the design of NMR contrast agents: manganese in blood as a model system. *Magnetic Resonance Med* **1984**;1:396–409
29. Castro ME, Boisvert DP, Treiber EO, Lunt JA, Allen PS. Presented at the annual meeting of the Society of Magnetic Resonance in Medicine, New York, August **1984**
30. Hopkins AL, Yeung HN, Bratton CB. Multiple field strength in vivo T1 and T2 for cerebrospinal fluid protons. *Magnetic Resonance Med* **1986**;3: 303–312
31. Vock P, Hedlund LW, Herfkens RJ, Effman EL, Brown MA, Putman CE. Work in progress. In vitro analysis of pleural fluid analogues by proton magnetic resonance—preliminary studies at 1.5T. *Invest Radiol* **1987**;22:382–387
32. Koenig SH. The dynamics of water-protein interactions. Results from measurements of nuclear magnetic relaxation dispersion. In: Rowland SP, ed. *Water in polymers*. Washington, DC: American Chemical Society, **1980**:157–175
33. Zipp A, James TL, Kuntz ID, Shohet SB. Water proton magnetic resonance studies of normal and sickle erythrocytes. *Biochim Biophys Acta* **1976**;28:291–303
34. Fullerton GD, Cameron IL, Ord VA. Frequency dependence of magnetic resonance spin-lattice relaxation of proton in biological materials. *Radiology* **1984**;151:135–138
35. Mather-DeVre R. Review article. Biomedical implications of the relaxation behavior of water related to NMR imaging. *Br J Radiol* **1984**;57:955–976
36. Barnhart JL, Berk RN. Influence of paramagnetic ions and pH on proton NMR relaxation of biologic fluids. *Invest Radiol* **1986**;21:132–136
37. Koenig SH, Brown RD, Lindstrom TR. Interactions of solvent and the heme region of methemoglobin and fluoro-methemoglobin. *Biophys J* **1981**;34:397–408
38. Axel L. Review. Blood flow effects in magnetic resonance imaging. *AJR* **1984**;143:1157–1166
39. Rubin JB, Enzmann DR. Harmonic modulation of proton MR precessional phase by pulsatile motion: origin of spinal CSF flow phenomena. *AJNR* **1987**;8:307–318