Rachael A. Clark, BS • Alyssa T. Watanabe, MD[2] • William G. Bradley, Jr, MD, PhD[3] • John D. Roberts, PhD

Acute Hematomas: Effects of Deoxygenation, Hematocrit, and Fibrin-Clot Formation and Retraction on T2 Shortening[1]

Acute hematomas can appear hypointense on T2-weighted magnetic resonance (MR) images at field strengths as low as 0.35 T. Using Raman spectroscopy to measure blood oxygenation and taking T2 measurements at 2.1 and 9.4 T, the authors examined the relaxation mechanisms acting during deoxygenation, increases in hematocrit, and fibrin-clot formation and retraction. Individual contributions to overall T2 from deoxyhemoglobin and the interactions of water with protein hydration layers in hemoglobin, plasma proteins, and fibrin were measured. Overall T2 values estimated by summing individual relaxation rates were in reasonable agreement with the T2 values of clotted blood. Results suggest that deoxygenation may be most important in T2 shortening, followed by increased hematocrit. T2 shortening from fibrin polymerization was minimal at the field strengths used. Effects of deoxygenation and increasing hematocrit are more sensitive to field strength than fibrin T2 shortening. Effects of fibrin may be more significant at middle and low field strengths.

Index terms: Blood, coagulation • Blood, MR studies • Brain, hemorrhage, 10.367, 10.43 • Brain, MR studies, 10.1214 • Hemoglobin • Magnetic resonance (MR), experimental • Magnetic resonance (MR), spectroscopy

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THE purpose of this study was to examine and quantitate the relaxation mechanisms underlying physiologic changes that are believed to cause enhanced T2 relaxation in acute hematomas.

BACKGROUND INFORMATION

Proton Populations in Blood

Water molecules in blood are divided into intracellular and extracellular populations by red cell membranes. Diffusion across red cell membranes appears to be reasonably rapid, and Andrasko (1) has calculated that the average lifetime of a water molecule inside an erythrocyte is 17 msec at 24°C. If there is fast exchange on the nuclear magnetic resonance (NMR) time scale between these two populations, a single transverse relaxation time is expected for the water protons.

A further subdivision of proton populations is necessary when blood is recognized as a protein solution. The primary protein component is hemoglobin, but there are others present in lesser concentrations, such as fibrinogen and lipoproteins. All of these proteins interact with water protons and affect the relaxation behavior of the water protons.

Protons in protein solutions can be divided on the basis of their mobility into three populations (2): (*a*) protons of water molecules that are not bound to protein (free water), (*b*) protons of water molecules in the protein hydration layers, and (*c*) covalently bound protons of protein molecules. The covalently bound protons of protein molecules have very short T2 relaxation times, and the spin-echo technique employed to measure T2 in this study effectively disregards signals from this group. Protons in the second group, those bound in the hydration layer, generally have the same translational and rotational mobility as the protein molecules themselves (3). Fast exchange between water in the protein hydration layers and free water will produce a single resultant relaxation time, which is a function of the proportion of water in the protein hydration layers and of the rotational correlation time characterizing the molecules of each of the kinds of proteins (2). The conditions of fast exchange predict that T2 will be linearly related to protein concentration, at least up to concentrations at which the proteins strongly interact with one another (3). We assume throughout that the T2 relaxation of the water in blood will be observed as a single exponential signal decay.

Factors That Can Cause T2 Relaxation in Acute Hematomas

The physiologic changes currently implicated in T2 shortening include deoxygenation of hemoglobin (4,5), increased hematocrit and hemoglobin concentration resulting from clot formation and retraction (6), and fibrin polymerization and clot retraction (7).

The hemoglobin in blood cells is expected to enhance transverse relaxation by two separate mechanisms. One is associated with paramagnetic deoxyhemoglobin formed in the deoxygenation of blood that will create

[1] From the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, CA 91126 (R.A.C., J.D.R.), and the MR Imaging Laboratory, Huntington Medical Research Institutes, Pasadena (A.T.W., W.G.B.). From the 1988 RSNA annual meeting. Received May 1, 1989; revision requested June 6; revision received November 6; accepted December 20. Supported by the California Institute of Technology Summer Undergraduate Research Fellowship Program and E. I. Du Pont. **Address reprint requests to** J.D.R.

[2] Current address: Department of Radiological Sciences, UCLA Medical Center, Los Angeles.

[3] Current address: Department of Radiology, Long Beach Memorial Medical Center, Long Beach, Calif.

Abbreviations: CPMG = Carr-Purcell-Meiboom-Gill, EDTA = ethylenediaminetetraacetic acid, UV = ultraviolet.

local magnetic gradients that act to dephase the proton moments in diffusing water molecules. The other involves water protons, which also will have shortened T2 values during the period that they are sequestered in the hydration layers of the protein.

Deoxyhemoglobin within red cells is expected to create an average difference in magnetic susceptibility between the interior and the exterior of the cells, although it has been reported that the effect is not more than 0.15 ppm (8). There will also be gradients within the cell, but their effects may be averaged by rapid diffusion of the water molecules. The precessing magnetic moments of water protons in different parts of these local field gradients will not stay in phase, and as diffusion occurs, unless it is fast, there will be further opportunities for dephasing, thus shortening T2 (4). This mode of proton relaxation is dependent on the square of the magnetic field strength, and Thulborn et al (4) expressed doubt that it would be significant at field strengths of 1.5 T or lower. However, Gomori et al (5) observed significant enhancement of T2 at 1.5 T using a long $2\tau_{CPMG}$, the Carr-Purcell-Meiboom-Gill interecho interval. Transverse relaxation was reported to be dependent on both the interecho interval and on the square of the field strength at 1.5 T. It was suggested that this mode of transverse relaxation depends on the paramagnetic hemoglobin being sequestered in red blood cells because, in these experiments, the T2 values of blood lysates were found to be independent of field strength and interecho interval.

Hemoglobin can also contribute to transverse relaxation by hydration-layer exchanges, as mentioned above. Hayman et al (6) found that lysing red cells does not, in fact, eliminate T2 shortening. These workers also established a linear relationship between $T2^{-1}$ and hematocrit for both oxygenated and deoxygenated blood. The part of the protein-hydration-layer relaxation mechanism, which is associated with the red cells, is expected to depend on hemoglobin concentration (and hematocrit) because it is dependent on the proportion of water in the hemoglobin hydration layers.

Other proteins in blood (eg, lipoproteins) also contribute to T2 relaxation. One of particular interest is fibrinogen, which, when converted to fibrin, polymerizes and forms a clot. Previous studies have shown that T2 decreases in fibrin solutions during the polymerization phase of clotting (2,3). Daszkiewicz et al (2) suggested that T2 shortening is the result of greatly increased correlation time of the water protons in the hydration layer of the fibrin molecules. The molecular correlation time reflects the rotational mobility of the fibrin molecules and would become larger with increasing length of the fibrin polymers and the increasing rigidity of the fibrin network (2).

When several relaxation mechanisms contribute to a single relaxation time, the overall T1 or T2 is related to the individual relaxation mechanisms by the following equation (9):

$$1/T2 = \Sigma 1/T2_i \qquad (1)$$

where the sum is over i individual relaxation mechanisms. Given that the mechanisms mentioned above are independent, we can predict the relaxation time of the water in a sample of clotted blood of known oxygenation and hematocrit by summing the individual relaxation rates:

$$1/T2_{obs} = 1/T2_{Hb\ par} + 1/T2_{Hb\ prot} + 1/T2_{fib\ prot} + 1/T2_{plas\ prot} \qquad (2)$$

where $1/T2_{Hb\ par}$ is the contribution characteristic of paramagnetic T2 shortening from diffusion through magnetic gradients in deoxyhemoglobin, $1/T2_{Hb\ prot}$ is the contribution associated with hemoglobin (oxy-, deoxy-, and methemoglobin) protein concentrations, $1/T2_{fib\ prot}$ is the contribution from polymerized fibrin molecules, and $1/T2_{plas\ prot}$ is the contribution characteristic of interactions with other plasma proteins.

In this study, we sought to isolate and measure each of these individual relaxation rates. We studied the behavior of T2 with deoxygenation, which was expected to change $1/T2_{Hb\ par}$ but have minimal effects on the other rates. We examined the dependence of T2 on hematocrit in both oxygenated and deoxygenated blood. Altering blood hematocrit changes the protein concentrations corresponding to hemoglobin and plasma proteins and also alters the distribution of the field and local field gradients, thus changing $1/T2_{Hb\ par}$, $1/T2_{Hb\ prot}$, and $1/T2_{plas\ prot}$ but leaving $1/T2_{fib\ prot}$ unaffected.

MATERIALS AND METHODS

The T2 values of samples of varying oxygenation, samples of varying hematocrit, and clotted samples were measured to determine the dependence of the transverse relaxation time on the degree of blood oxygenation, the hematocrit, and presence of clotting.

Sample Preparation

Blood was obtained from human volunteers and stored for a few hours at 4°C until used.

Samples of varying hematocrit.—Hematocrit was measured by centrifuging samples and visually measuring the proportion of red blood cells to total sample volume. The hematocrit was increased by centrifuging samples and removing the supernatant plasma. Samples with lower hematocrit were prepared by mixing fractions of whole blood and plasma.

Samples of varying oxygenation.—Blood was deoxygenated completely by adding sodium dithionite, 5 mg/mL, and stored under argon. Oxygenation was increased by incubating untreated blood in an atmosphere of molecular oxygen. Glassware was coated with paraffin to minimize red cell lysis. Samples were stored at 4°C between measurements of T2 and oxygenation.

Samples with and without clots.—Plasma samples with and without buffy coat were clotted to determine the relaxation rate associated with fibrin polymerization. Samples without buffy coat were clotted because such samples undergo normal clot formation but contain far fewer platelets (therefore there is little clot retraction), allowing us to estimate the relative contributions of fibrin polymerization and clot retraction to overall fibrin T2 shortening.

Heparinized blood was clotted in NMR tubes for clotting experiments with use of 0.2 mg protamine per milliliter of whole blood and 0.04 mL reconstituted bovine thrombin per milliliter of whole blood. Only samples with well-formed clots were used.

Plasma with buffy coat was obtained by removing plasma from above settled red cells. The buffy coat was removed from plasma by centrifugation to produce plasma clots free of all cellular elements.

Clots of whole deoxygenated blood were made from samples treated with sodium dithionite. Clots of oxygenated blood were made from whole blood samples incubated for 1.5 hours under oxygen at 25°C. The T2 values of samples of clotted oxygenated and deoxygenated blood were compared with those calculated with use of the individual rates mentioned in the Background section.

Determination of Hemoglobin Oxygenation

Blood oxygenation was determined by means of a new quantitative application of Raman spectroscopy. Raman spectroscopy, a light-scattering technique commonly used to probe the electronic structure of complex molecules, examines the coherent laser light scattered by a sample.

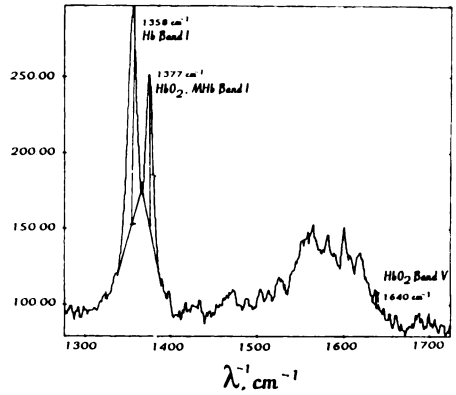


Figure 1. Laser Raman spectrum of a whole blood sample. Oxygenation of samples was measured by comparing the peak intensities of band *I*, a hemoglobin oxidation-state marker band, with band *V*, a spin-state marker band. Sample shown contains 77% oxyhemoglobin, 33% deoxyhemoglobin, and less than 1% methemoglobin. The laser wavelength was 441.6 nm. *Hb* = deoxyhemoglobin, HbO_2 = oxyhemoglobin, *MHb* = methemoglobin.

Some of the incident photons scattered by the sample lose or gain energy. Examining these scattered photons of altered energy can provide detailed information as to the electronic and vibrational structures of the sample molecule. Laser Raman spectroscopy differs from ultraviolet (UV) spectroscopy in that the Raman effect involves light scattering, instead of light absorption. In UV spectroscopy, photons with frequencies corresponding to energy differences in the molecule are absorbed, and the molecule is raised to an excited state. The excited molecule can later lose its excitation energy through radiation or other mechanisms. In the Raman effect, the incident photons are scattered, not absorbed, and the frequencies of the incident photons have no relationship to energy differences of the molecule.

Oxy-, deoxy-, and methemoglobin each have distinctive Raman marker bands (Fig 1) with laser Raman irradiation at 441.6 nm. Band I, an oxidation-state marker band, is thought to reflect the electron occupancy of the porphyrin π^* orbitals. High occupancy of these orbitals weakens the porphyrin bonds and decreases their vibrational frequency (10–12). Band I appears at 1,358 cm^{-1} for deoxyhemoglobin and at 1,377 cm^{-1} for oxy- and methemoglobin. Band V, a spin-state marker band, reflects changes in expansion of the porphyrin core (13,14). Band V of oxyhemoglobin occurs at 1,640 cm^{-1}.

Spectra of pure oxy-, deoxy-, and methemoglobin were used to measure intensities of bands I and V at 100% oxygenation, deoxygenation, and oxidation. Intensities of bands I and V in pure hemoglobin samples, measured relative to a 0.4 mol/L Na_2SO_4 standard, are shown in the Table. Oxygenation of test samples was measured by comparing the intensities of bands I and V to their known maximum intensities from the spectra of pure hemoglobin standards. That is, maximum peak heights for both bands were measured on samples of 100% oxy-, deoxy-, and methemoglobin, and the percent maximum intensity of these bands in the test sample was directly related to the percent oxygenation.

Comparison of band I intensity for deoxyhemoglobin (1,358 cm^{-1}) relative to the intensity of oxyhemoglobin band V (1,640 cm^{-1}) yields *A*, the mole fraction of deoxygenated blood divided by the mole fraction of oxygenated blood:

$$A = \frac{1}{35.7} \times \frac{I_{1,358}}{I_{1,640}} = \frac{\text{mole fraction deoxyhemoglobin}}{\text{mole fraction oxyhemoglobin}}, \quad (3)$$

where $I_{1,358}$ = intensity at 1,358 cm^{-1}, $I_{1,640}$ = intensity at 1,640 cm^{-1}, and 35.7^{-1} is a standardization factor that relates the intensity of deoxyhemoglobin band I at 100% deoxygenation to that of oxyhemoglobin band V at 100% oxygenation.

Likewise, the relative intensities of bands I (oxy- and methemoglobin, 1,377 cm^{-1}) and band V (oxyhemoglobin, 1,640 cm^{-1}) minus the maximum intensity of band I at 100% oxygenation (2.13) is equal to the mole fraction of methemoglobin divided by the mole fraction of oxyhemoglobin:

$$B = 0.28 \frac{I_{1,377}}{I_{1,640}} - 2.13 = \frac{\text{mole fraction methemoglobin}}{\text{mole fraction oxyhemoglobin}}. \quad (4)$$

We then have

$$\text{mole fraction oxyhemoglobin} = \frac{1}{A + B + 1}, \quad (5)$$

$$\text{mole fraction deoxyhemoglobin} = A \times \text{oxyhemoglobin fraction}, \quad (6)$$

and

$$\text{mole fraction methemoglobin} = B \times \text{oxyhemoglobin fraction}. \quad (7)$$

The relative intensities of Raman bands were determined by measuring the peak heights of plotted spectra (Fig 1). The intensity of band V of oxyhemoglobin is very small, especially in samples consisting mainly of deoxyhemoglobin (Fig 1). Care must be taken to minimize noise on the Raman spectrum so that this peak can be accurately measured. Noise can be reduced by taking several scans and by using dilute samples (samples of low hematocrit). Ethylenediaminetetraacetic acid (EDTA) is the preferred anticoagulant in these experiments, because heparinized samples fluoresced at 441.6 nm. However, it was necessary to use heparin as an anticoagulant when the blood was clotted with protamine. For these cases, the degree of oxygenation was measured by taking two identically prepared samples; one with EDTA was used to measure the initial oxygenation level while the heparinized sample was clotted.

Previous studies have used UV absorption to determine oxygenation of blood samples (4,5). We chose not to use UV spectroscopy because it would require transferring oxygen-sensitive samples from NMR tubes to cuvettes. Raman spectroscopy allowed us to make both T2 and oxygenation measurements on the same sealed capillary tube. In all experiments, T2 was measured before Raman spectroscopy because exposure of blood to laser light causes photolysis of hemoglobin. Changes in blood oxygenation were minimized by allowing less than 1 hour between measurements.

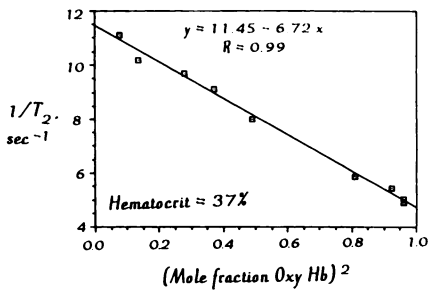
Measurement of Transverse Relaxation Time

Measurements of the proton T2 relaxation time were made on 89.55-MHz and 399.65-MHz Fourier-transform NMR spectrometers (Jeol Ltd, Tokyo) operating at 2.1 and 9.4 T, respectively. A CPMG sequence was used (15): $90_x - (\tau_{CPMG} - 180_y - \tau_{CPMG})_n$. We restricted $2\tau_{CPMG}$ to short values (less than 6 msec for the 89.55-MHz measurements and 2 msec for the 399.65-MHz measurements) to avoid possible distortion of the results by the inhomogeneities of the magnetic fields of our spectrometers. Intensities of NMR water resonance peaks were recorded at even values of $2\tau_{CPMG}$, and T2 was calculated by a least-squares fit to a single exponential. Only measurements that had standard deviations of less than 5% from the least-squares fit were used. Duplicate T2 measurements were taken of all samples; plotted values are the average of the two measurements. Standard deviations of duplicate readings were within 3% of the T2 for the plasma-clot samples and within 5% for all other samples. Clotting samples were often observed to have two peaks with distinctive relaxation times. The slowly relaxing component had relaxation times characteristic of plasma and were judged to arise from plasma surrounding the retracted clot. The shorter relaxation times were believed to be from water within the clot; these shorter relaxation times are reported here as T2 of the clot. All measurements were made at 37°C.

RESULTS

Dependence of T2 on Oxygenation at 90 MHz

Raman measurements showed that there was 1% or less methemoglobin in all samples of oxy- and deoxyhemoglobin. Figure 2 shows a linear relationship between $T2^{-1}$ and the square of the mole fraction of oxyhemoglobin, with a correlation coefficient of .99.



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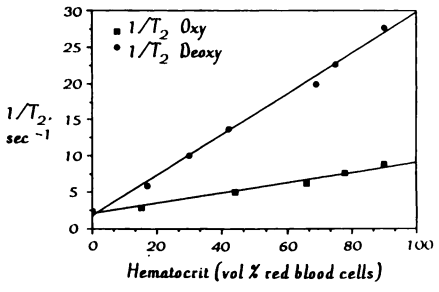

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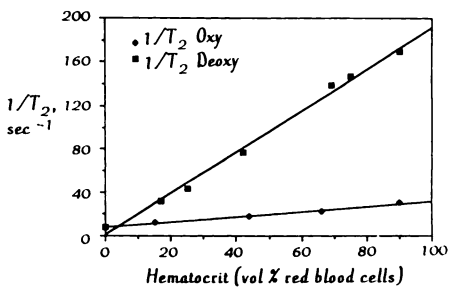

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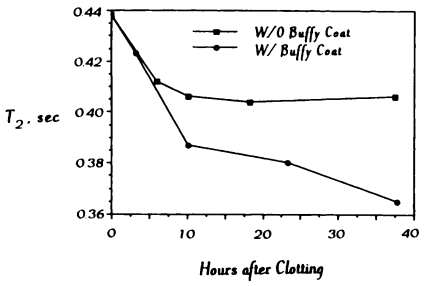
Figures 2-4. **(2)** The linear relationship between $T2^{-1}$ and the square of the mole fraction of oxyhemoglobin at 90 MHz (2.1 T). $2\tau_{CPMG}$ = 6 msec. Hematocrit was 37%. Other workers (4) have reported a linear relationship between $T2^{-1}$ and the square of the mole fraction of deoxyhemoglobin. **(3)** Linear correlation of T2 with hematocrit at 90 MHz. $2\tau_{CPMG}$ = 4 msec. The minimum T2 is at a hematocrit of 100% for both deoxy- and oxyhemoglobin. **(4)** Linear correlation of T2 with hematocrit at 400 MHz (9.4 T). $2\tau_{CPMG}$ = 2 msec.

Raman-Band Intensities of Pure Hemoglobin Samples Relative to 0.4 mol/L Na_2SO_4 Standard at 441.6 nm

	Hemoglobin			
$\Delta\nu$	Deoxy-	Oxy-	Met-	Band Assignment
1,358	10.0	...	...	I
1,377	...	2.13	1.63	I
1,640	...	0.28	...	V

Note.—Values are $I_\nu/I_{Na_2SO_4}$ (I = intensity). Values of $\Delta\nu$ are expressed in wave numbers.



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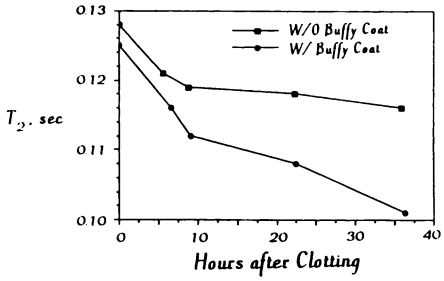

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Figures 5, 6. **(5)** Changes in T2 of clotting plasma with and without buffy coat at 90 MHz. $2\tau_{CPMG}$ = 4 msec. Zero-hour reading was taken immediately after adding protamine and thrombin. **(6)** Changes in T2 of clotting plasma at 400 MHz. $2\tau_{CPMG}$ = 2 msec.

Dependence of T2 on Hematocrit in Oxygenated and Deoxygenated Blood

Raman measurements confirmed that 99% or more of dithionite-treated blood hemoglobin was in the deoxygenated form. Raman measurements of oxygenated samples showed that they contained 96% or more oxyhemoglobin. Figures 3 and 4 show linear relationships between hematocrit and $T2^{-1}$ obtained at 89.55 and 399.65 MHz for both oxy- and deoxyhemoglobin. Correlation constants for all four linear fits were .99. The linear plots of Figures 3 and 4 are in accord with the results of Hayman et al (6).

Dependence of T2 on Clotting

Plasma clots.—The T2 of plasma clots with buffy coat decreased from 438 to 365 msec at 89.55 MHz and from 125 to 101 msec at 399.65 MHz within 38 hours after adding protamine and thrombin (Figs 5, 6). At both field strengths, the T2 of plasma clots without buffy coat decreased by roughly half the above amount. A plasma sample with 2.5–3.0 times the normal concentration of buffy coat underwent a 54% decrease in T2 over 24 hours.

Oxyhemoglobin clots.—The T2 of oxyhemoglobin clots decreased from 197 to 145 msec within 37 hours after clotting at 89.55 MHz (Fig 7). Similarly, T2 at 399.65 MHz decreased from 57 to 46 msec within 37 hours (Fig 8). No red cell lysis was observed in these experiments, and the intense red color of the clots did not change, indicating no significant conversion to deoxy- or methemoglobin.

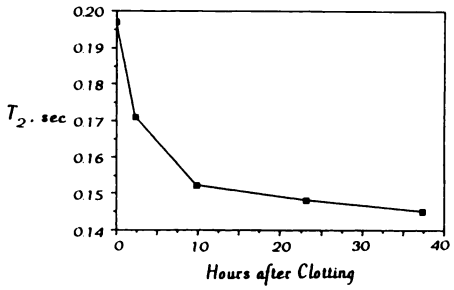
Deoxyhemoglobin clots.—At 89.55 MHz, T2 decreased from 73 to 31 msec by 38 hours after clotting (Fig 9), while at 399.65 MHz, the decrease was from 12.9 to 6.2 msec (Fig 10). No red cell lysis or color change was observed during these experiments.

DISCUSSION

Equations (1) and (2) express the overall T2 relaxation time of clotted blood in terms of rates of individual relaxation mechanisms. If we have properly quantitated the rates of the major relaxation mechanisms responsible for T2 shortening, summation of the individual rates should produce overall T2 values for clotted blood samples that are in reasonable agreement with the experimentally measured T2 values.

Measurement of $1/T2_{plas\ prot}$ is straightforward. To obtain a sample with a hematocrit of 0%, red blood cells were removed by centrifugation, leaving plasma proteins in solution. The T2 of this 0%-hematocrit sample is determined by interactions of water with plasma proteins; thus, $1/T2_{plas\ prot}$ is equal to 2.28 sec^{-1} at 90 MHz. This rate includes contributions from lipoproteins and fibrinogen, the unpolymerized precursor of fibrin. Because fibrinogen is included in the first term, $1/T2_{fib\ prot}$ is 0 in unclotted samples.

Clotted samples of plasma with and without buffy coat were prepared to measure $1/T2_{fib\ prot}$. Samples with buffy coat (containing normal concentrations of plasma proteins, platelets, and fibrinogen) showed a 16%–17% decrease in transverse relaxation time to 0.37 seconds by 38 hours after clotting. If we assume that the degree of fibrin polymerization after 38 hours is the same as would be observed in a whole blood clot over this period, then the small relaxation contribution from fibrin polymerization (38 hours after clotting) is given by the equation $1/T2_{fib\ prot} = (1/0.37) - 1/T2_{plas\ prot} = 0.42\ sec^{-1}$ at 90 MHz. $1/T2_{plas\ prot}$ is subtracted from this rate because plasma proteins not participating in clotting also influence the relaxation rate in these samples. We recognize that the value of $1/T2_{plas\ prot}$ contains a contribution from the unpolymerized fibrinogen molecules. Clearly, when these molecules are converted to fibrin and form a clot, they no long-



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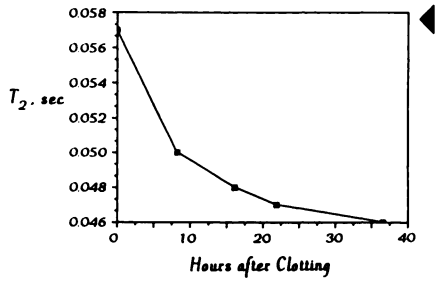

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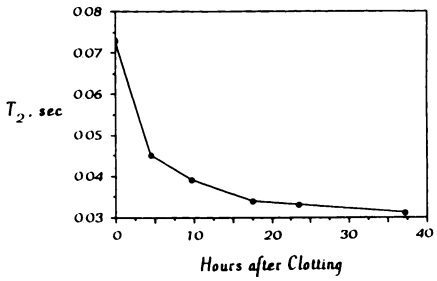
Figures 7, 8. **(7)** Changes in T2 of clotting oxygenated whole blood at 90 MHz. $2\tau_{CPMG}$ = 4 msec. Zero-hour reading was taken immediately after adding protamine and thrombin. **(8)** Changes in T2 of clotting oxygenated whole blood at 400 MHz. $2\tau_{CPMG}$ = 2 msec.



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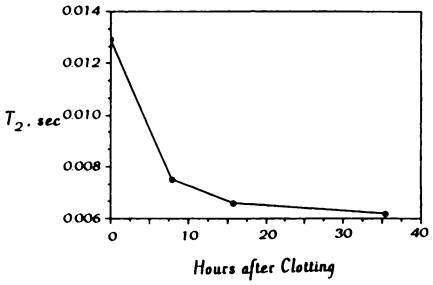

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Figures 9, 10. **(9)** Changes in T2 of clotting deoxygenated whole blood at 90 MHz. $2\tau_{CPMG}$ = 4 msec. **(10)** Changes in T2 of clotting deoxygenated whole blood at 400 MHz. $2\tau_{CPMG}$ = 2 msec.

er contribute to $1/T2_{plas\ prot}$. Thus, $1/T2_{plas\ prot}$ will decrease when clotting occurs. We have neglected this decrease in calculating the relaxation rate from fibrin polymerization, expecting that because fibrinogen is highly mobile with a relatively short correlation time, it should not greatly affect T2. On the other hand, networks of fibrin polymers should greatly affect T2 because of their long correlation times. For these reasons, the T2 change from fibrin polymerization is probably large enough to render the small error in its calculation relatively insignificant.

The two rate constants for relaxation via hemoglobin paramagnetic and protein effects, $1/T2_{Hb\ par}$ and $1/T2_{Hb\ prot}$, remain to be determined. Whole blood is a mixture of plasma and red blood cells. The hematocrit, the percentage of the total volume occupied by red blood cells, expresses the relative abundance of each fraction. There are two major changes in the transverse relaxation rate as the hematocrit increases. First, the contribution of plasma proteins to the total relaxation time decreases as the solution becomes more concentrated in red blood cells. The change in contribution of the plasma proteins is expected to lead to an approximately linear decrease of $1/T2_{plas\ prot}$ with increasing hematocrit. Second, the increasing concentrations of red blood cells in samples of increased hematocrit shorten T2 by both paramagnetic effects (with deoxyhemoglobin) and protein effects (with oxy- and deoxyhemoglobin). Because diamagnetic effects are usually small, it seems reasonable to assume that the diamagnetic effects of deoxy- and oxyhemoglobin are at least comparable. The relationships of 1/T2 to hematocrit can then be formulated as follows. For deoxygenated, unclotted blood,

$$1/T2 = (1/T2_{plas\ prot})[(1 - Hct)/100] + (1/T2_{Hb\ par} + 1/T2_{Hb\ prot}) \times (Hct/100), \quad (8)$$

and for oxygenated, unclotted blood,

$$1/T2 = (1/T2_{plas\ prot})[(1 - Hct)/100] + (1/T2_{Hb\ prot})(Hct/100), \quad (9)$$

where $1/T2_{plas\ prot}$ is the relaxation rate of the 0%-hematocrit (Hct) sample from Figures 3 and 4 (2.28 sec^{-1} at 90 MHz) and $(1/T2_{Hb\ par} + 1/T2_{Hb\ prot})$ is the relaxation rate of the deoxygenated 100%-hematocrit sample (29.6 sec^{-1} at 90 MHz). The constant $1/T2_{Hb\ prot}$ contains small contributions to relaxation from differences in diamagnetic susceptibilities. Oxyhemoglobin is not paramagnetic, so $1/T2_{Hb\ par}$ is equal to 0 for these samples.

Estimated versus Observed T2 for Deoxygenated Clots

Clots of 99% deoxygenated whole blood retracted to half their original volume by 37 hours after clotting. The starting hematocrit was approximately 45%; clotting and retraction produced a clot with a hematocrit of approximately 90% (45 ÷ 50).

The estimated overall T2 ($T2_{est}$) was obtained as follows: $1/T2_{est} = 1/T2_{unclotted} + 1/T2_{fib\ prot}$ = 27.3 sec^{-1} at 90 MHz ($1/T2_{unclotted}$ comes from Eq [8]). This gives $T2_{est}$ as 37 msec, in good agreement with 35 msec observed for T2 at 38 hours after clotting. A similar calculation with use of the data taken at 400 MHz yields an estimated T2 of 5.7 msec, and the observed T2 was 6.2 msec (Fig 10).

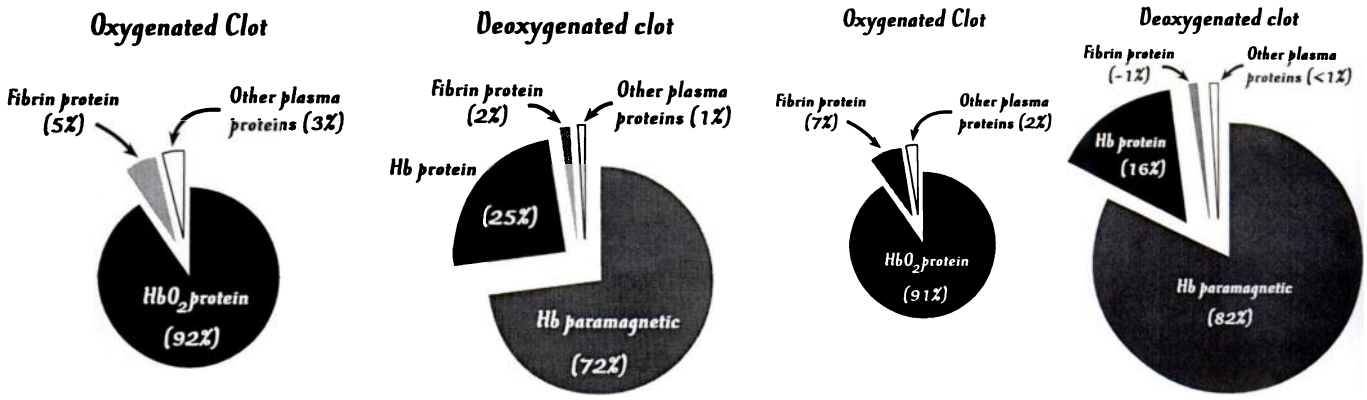
Estimated versus Observed T2 for Oxygenated Clots

Clots of 96% oxygenated whole blood retracted roughly the same amount as did those of deoxygenated blood, and the approximate hematocrit was 90% after 37 hours.

The estimated T2 was as follows: $1/T2_{est} = 1/T2_{unclotted} + 1/T2_{fib\ prot}$ = 7.67 sec^{-1} at 90 MHz ($1/T2_{unclotted}$ comes from Eq [9]). The $T2_{est}$ is 130 msec, in reasonable agreement with the 145 msec observed for T2 of oxygenated clots at 38 hours after clotting (Fig 7). At 400 MHz, the estimated T2 was 31 msec, and the observed value is in poor agreement at 46 msec.

Figures 11 and 12 show estimates of the proportional contribution to the overall T2 rate from each individual relaxation rate at 90 and 400 MHz. Contributions were calculated as percentages of the total rate.

As shown in Figures 11 and 12, with the high hematocrits characteristic of retracted blood clots (greater than 90%), hemoglobin protein is mainly responsible for relaxation enhancement in both oxygenated and deoxygenated blood at both field strengths we used. Also, deoxygenation of blood is more important to T2 shortening than the protein effects of both oxy- and deoxyhemoglobin at these field strengths; the contribution from the $1/T2_{Hb\ par}$ relaxation rate, calculated by subtracting the oxyhemoglobin relaxation rate from the deoxyhemoglobin rate, was 72% of the overall rate at 90 MHz and 82% at 400 MHz. The contributions to relaxation in oxygenated clots were the same (to within 2%) at both field strengths, as were the fibrin and plasma protein rates in deoxygenated clots. The only major difference between the two field strengths was the increased paramagnetic contribution at 400 MHz; the paramagnetic T2 shortening of deoxyhemoglobin depends on the square of the magnetic field strength, and as expected, its contribution increases with field strength.



11. 12.

Figures 11, 12. Pie charts show estimates of the proportional contributions of the individual relaxation rates associated with the paramagnetism of deoxyhemoglobin and the concentrations of hemoglobin, fibrin, and plasma proteins to the overall T2 relaxation rate at 90 **(11)** and 400 **(12)** MHz for oxygenated and deoxygenated clotted blood. The sizes of the pies are not to scale but are drawn differently to emphasize that the total relaxation rates are not the same for oxygenated and deoxygenated clots.

CONCLUSIONS

In clinical practice, acute intracerebral hemorrhage may appear hypointense on T2-weighted images at field strengths as low as 0.35 T (16). Of the physiologic changes that we evaluated that could act to shorten the T2 of acute hematomas—deoxygenation of blood, increase in hematocrit, and fibrin-clot formation and retraction—deoxygenation appears to be the most important. Obviously, the T2 change arising from deoxygenation will depend on the degree to which the blood becomes deoxygenated and on the field strength.

The T2 values of blood are also strongly affected by hematocrit, and the increased hematocrit resulting from clot retraction in hematomas can be expected to shorten T2 significantly.

Fibrin polymerization and clot retraction had small effects on T2 at the field strengths used in this study. Approximately half of the fibrin-induced T2 shortening appeared to result from clotting and half from clot retraction. Although the T2 shortening from clotting was quite small in our experiments, it appears to be sensitive to platelet concentration. Not every hematoma clots and retracts, and those that do may differ in platelet concentration, the degree of clotting, and the amount of clot retraction. Consequently, some degree of uniqueness must be expected in the overall T2 decrease for different hemorrhages.

Because the effects on T2 from deoxygenation and increasing hematocrit are more sensitive to field strength than are those arising from fibrin-clot formation and retraction, the latter may be relatively more important causes of T2 shortening in acute hematomas at middle and low field strengths.

In this study T2 was measured with short interecho times (4–6 msec). Clinical imagers commonly use interecho (ie, echo delay) times of around 20–40 msec. With long interecho times, the T2 shortening resulting from diffusion through localized magnetic gradients in deoxygenated blood should increase. Gomori et al (5) reported that the apparent values of T2 from intracellular deoxyhemoglobin decreased by a factor of three when the interecho time was increased from 4 to 64 msec. However, it is not completely clear how much of the reported decrease was caused by diffusion in the local gradients associated with hemoglobin and how much could be ascribed to inhomogeneities in the static magnetic field. Irrespective of this, it is clear that in applying the results of the present study to clinical imaging, differences in interecho times must be taken into account. ■

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References

1. Andrasko J. Water diffusion permeability of human erythrocytes studied by a pulsed gradient NMR technique. Biochim Biophys Acta 1976; 428:304–311.
2. Daszkiewicz OK, Jedrychowski A, Kirchmayer S. Studies on blood clotting by the spin echo technique. Acta Med Pol 1965; 1:95–102.
3. Jedrychowski A. Nuclear magnetic resonance studies of fibrin polymerization. Thromb Diath Haemorrh 1968; 20:513–527.
4. Thulborn KR, Waterton JC, Matthews PM, Radda GK. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. Biochim Biophys Acta 1982; 714:265–270.
5. Gomori JM, Grossman RI, Yu-Ip C, Asakura T. NMR relaxation times of blood: dependence on field strength, oxidation state, and cell integrity. J Comput Assist Tomogr 1987; 11:684–690.
6. Hayman LA, Ford JJ, Taber KH, Saleem A, Round ME, Bryan RN. T2 effect of hemoglobin concentration: assessment with in vitro MR spectroscopy. Radiology 1988; 168:489–491.
7. Hayman LA, Taber KH, Ford JJ, Saleem A, Gurgun M, Mohamed S, Bryan RN. Effect of clot formation and retraction on spin-echo MR images of blood: an in vitro study. AJNR 1989; 10:1155–1158.
8. Brunetti A, Alger J, Brooks R, Di Chiro G. On the origin of paramagnetic inhomogeneity in blood (abstr). In: Book of abstracts: Society of Magnetic Resonance in Medicine 1988. Vol 1. Berkeley, Calif: Society of Magnetic Resonance in Medicine, 1988; 233.
9. Levy GC. Carbon-13 nuclear spin relaxation. In: Topics in carbon-13 NMR spectroscopy. Vol 1. New York: Wiley, 1974; 96.
10. Kitagawa T, Iizuka T, Saito M, Kyogoku Y. Resonance Raman scattering from hemoproteins: the nature of the bond between the sixth ligand and the heme iron in ferrous low-spin derivatives of hemoglobin. Chem Lett 1975; 849–852.
11. Kitagawa T, Ozaki Y, Kyogoku Y. Resonance Raman studies on the ligand-iron interactions in hemoproteins and metalloporphyrins. Adv Biophys 1978; 11:153–196.
12. Spiro TG, Stong JD, Stein P. Protein control of porphyrin conformation: comparison of resonance Raman spectra of heme proteins with mesoporphyrin IX analogues. J Am Chem Soc 1976; 98:5482–5489.
13. Lanir A, Yu NT, Felton RH. Conformational transitions and vibronic couplings in acid ferricytochrome c: a resonance Raman study. Biochem 1979; 18:1656–1660.
14. Spiro TG, Stong JD, Stein P. Porphyrin core expansion and doming in heme proteins: new evidence from resonance Raman spectra of six-coordinate high-spin iron(III) hemes. J Am Chem Soc 1979; 101:2648–2655.
15. Meiboom S, Gill D. Modified spin-echo method for measuring nuclear relaxation times. Rev Sci Instr 1958; 29:688–691.
16. Bradley WG. MRI of hemorrhage and iron in the brain. In: Stark DD, Bradley WG, eds. Magnetic resonance imaging. St Louis: Mosby, 1988; 359–374.