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Effect of Clot Formation and Retraction on Spin-Echo MR Images of Blood: An in Vitro Study

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Phantoms were constructed that contained red blood cell (RBC)-free clots in varying stages of clot retraction. MR images of these samples were compared with those of retracted whole venous blood clots and a fresh rat brain standard. Images were obtained at 0.3 T, 0.5 T, 1.0 T, 1.5 T, and 2.4 T with T1-, spin-density- and T2-weighted spin-echo pulse sequences. The presence or absence of venous blood cells in the clot caused only minor differences in T2- and spin-density-weighted images of the clots at or below 1.5 T. On T2-weighted scans, the retraction of the RBC-free clot resulted in a progressive decrease in signal intensity at 2.4 T. Fully retracted RBC-free clots were markedly hypointense relative to serum and ranged from slightly hyperintense to isointense with brain and venous clots at 0.5–1.5 T. There were no striking concomitant signal intensity changes on the spin-density- or T1-weighted scans, which could have caused the changes seen on the T2-weighted images of the clots.

Our results indicate that the physical basis of these MR effects in the RBC-free clots is the concentration of plasma protein. The combined concentration of plasma protein and the tightly packed RBC proteins in the venous clots causes the strikingly similar MR appearance of venous and RBC-free clots on clinical images. These results do not demonstrate the presence of the previously postulated selective T2 relaxation of intracellular paramagnetic deoxyhemoglobin in these in vitro venous clots.

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Acute intracerebral hemorrhage contains blood in varying stages of clot formation and retraction [1]. Previous research to uncover the basis of the MR signal from these regions used red blood cells (RBCs) suspended in plasma, rather than clotted blood samples. In reports of these studies it was postulated that intracellular paramagnetic species, such as deoxyhemoglobin and methemoglobin, caused a selective reduction in T2 relaxation. This was ascribed to water molecules diffusing across gradients generated by packaging the paramagnetic species intracellularly [2]. This selective relaxation requires the presence of an extracellular compartment lacking paramagnetic agent(s), as well as intracellular paramagnetic agent(s). However, the number and mobility of the extracellular water molecules are altered by clot formation. In addition, when platelet function is normal, RBCs trapped within the clot are packed tightly together. These cellular zones have a greatly increased concentration of intracellular and extracellular protein. They are surrounded by lakes of serum that vary in size depending on the degree of clot contraction.

The present study was undertaken to determine the effects of clot formation and retraction on spin-echo MR images. The appearance of RBC-free clots was compared with venous blood clots to assess the relative contribution of the RBCs to the MR appearances obtained at a range of field strengths.

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Materials and Methods

Sample Preparation

RBC-free plasma was produced from anticoagulated human blood, which was drawn by venipuncture, centrifuged at 100 G for 10 min, and had the RBC-free supernatant removed. To form an RBC-free retracted clot, bovine thrombin (10 units/ml plasma) was added to this supernatant. Platelet-depleted plasma was formed by centrifuging the RBC-free plasma at 1000 G for 30 min and removing the platelet-depleted supernatant. An RBC-free unretracted clot was formed by adding bovine thrombin (10 units/ml plasma) to this supernatant.

An additional sample of human blood was obtained by venipuncture. To maximize the deoxyhemoglobin concentration in the blood, the donor was asked to exercise his arm with the tourniquet in place. The blood was drawn into a 50-ml plastic syringe after a color change was noted in the distal tissues. A 1-ml aliquot was immediately placed in an airtight heparinized plastic syringe and kept at room temperature. The pH, pCO_2, and pO_2, in mm Hg, were measured directly from the syringe by using a Radiometer ABL3* within 1 hr. The remainder of the venous blood sample was placed in clean glass containers and allowed to clot for 24 hr at room temperature (approximately 24°C). A freshly excised (0–10 hr) intact rat brain was placed in serum or in an isotonic saline solution or wrapped in plastic to prevent dehydration. It was included as an intensity standard in all phantoms.

MR Imaging

Scans were obtained by using a 3–5-mm slice thickness with 2–4 excitations, and the smallest possible field of view, which ranged from 12–19 cm depending on the scanner. The samples were at room temperature and undisturbed during the imaging. The images were filmed at identical window settings to allow accurate comparisons between serial scans (study 1) or in sequences that varied the TE (studies 2 and 3).

Study 1

Serial T2-weighted, single-slice, spin-echo images (6000/90/2–4) were obtained on a 2.4-T Bruker Biospec 24/40 system from a phantom (IA) that contained a rat brain standard and an RBC-free retracted clot. Thrombin was added to platelet-rich plasma immediately prior to image collection to acquire data in the earliest stages of RBC-free clot retraction. The initial image was completed within 0.5 hr after the addition of thrombin. The final image set was started 4 hr later. This experiment was repeated in a second identical phantom (IB) in which region of interest measurements were performed on each sample. T1- or spin-density (SD)-weighted pulse sequences were not done on either of these phantoms.

Study 2

T1-, T2-, and SD-weighted spin-echo images were obtained of a phantom (II) that contained retracted and unretracted RBC-free clots, whole venous blood clot, heparinized blood (Hct = 42%), which was frozen and thawed to induce blood cell lysis, and a rat brain standard. Images were obtained first on a 1.5-T GE system at which time clots were 24 hr old and brain and blood were 3 hr old. Images were obtained 12 hr later on 0.5-T and 1.0-T Seimens systems (see Table 1 for details of acquisition parameters).

Study 3

T1-, T2-, and SD-weighted single-slice spin-echo images were obtained on a 0.3-T Bruker BMT 1100 system of a phantom (III) that contained an RBC-free retracted clot (24 hr old), a retracted whole venous blood clot (22 hr old), and a fresh rat brain standard. The details of the imaging sequences are given in Table 1. Visual observation of sample signal intensities was confirmed by region of interest values.

* Radiometer, Copenhagen, Denmark.

Results

Blood drawn by venipuncture for the MR phantoms had measured pO_2 values in the range of 26–38 mm Hg (30–50% deoxyhemoglobin saturation). These levels of oxygenation extend below the normal pO_2 of 40 mm Hg, which has been measured in venous blood returning from tissues at rest. The pH values in the lysed blood samples ranged from 7.25 to 7.28, and in the clotted blood they ranged from 7.3 to 7.4.

Study 1 (Fig. 1)

The RBC-free clot formed in the platelet-rich plasma was virtually completely retracted 4 hr after the introduction of thrombin. At that time the clot had contracted to less than 25% of the volume of the container. This change in size was clearly delineated on the MR scans. The signal intensity of the retracting clot on the T2-weighted spin-echo scans dropped steadily. By 4 hr the RBC-free clot was very hypointense relative to serum and slightly hyperintense relative to the rat brain standard on region of interest measurements.

Study 2 (Fig. 2)

The fully retracted 24-hr-old RBC-free clot, the whole blood venous clot, and the rat brain were all isointense on the T2-weighted spin-echo scans at all field strengths. The serum and lysed venous blood samples were hyperintense compared with brain and clotted samples on the T2-weighted images. The unretracted gelatinous RBC-free clot was mildly hypointense relative to serum and markedly hyperintense relative to brain and all other samples on T2-weighted images. Similar findings were present on the SD-weighted images at all three field strengths, but the differences among samples were much smaller.

At 0.5 T, 1.0 T, and 1.5 T on T1-weighted scans lysed blood, whole venous blood clot, and brain were isointense. The retracted and unretracted RBC-free clots were isointense with the surrounding sera and hypointense relative to all other samples.

Study 3 (Fig. 3)

The 24-hr-old retracted RBC-free clot, whole venous blood clot, and brain had virtually identical signal intensities on SD-weighted images. On the longer TE scans from the multiecho sequence, which had progressively more T2 influence, the brain was slightly hyperintense relative to the RBC-free and whole venous blood clots.

TABLE 1: Acquisition Parameters* Used to Evaluate Phantoms II and III

Spin-Echo Pulse Sequences	Field Strengths			
	0.3 T	0.5 T	1.0 T	1.5 T
T1-weighted	500/34	300/35	300/35	600/20
T2-weighted	3000/134	3000/105	3000/105	4000/120
Spin-density-weighted	3000/34	3000/35	3000/35	4000/20

* Parameters given are TR/TE.

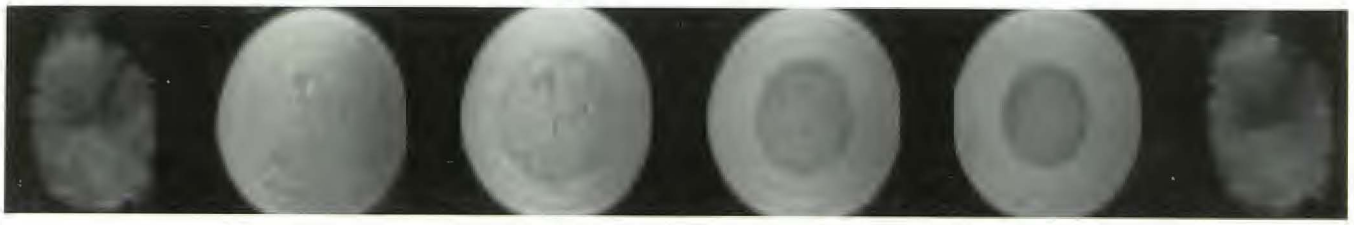

Fig. 1.—Phantom IB. 2.4-T serial spin-echo scans (6000/90) of platelet-rich RBC-free plasma obtained 1.3, 2.3, 3.3, and 4 hr, respectively, after addition of thrombin to initiate clot retraction. Images at extreme right and left are brain standards. The brain standards and RBC-free samples were all photographed with identical window settings to demonstrate the temporal reduction in T2 signal that occurred as clot retraction progressed. Region of interest measurements of the RBC-free clot at 4 hr were only slightly hyperintense relative to brain.

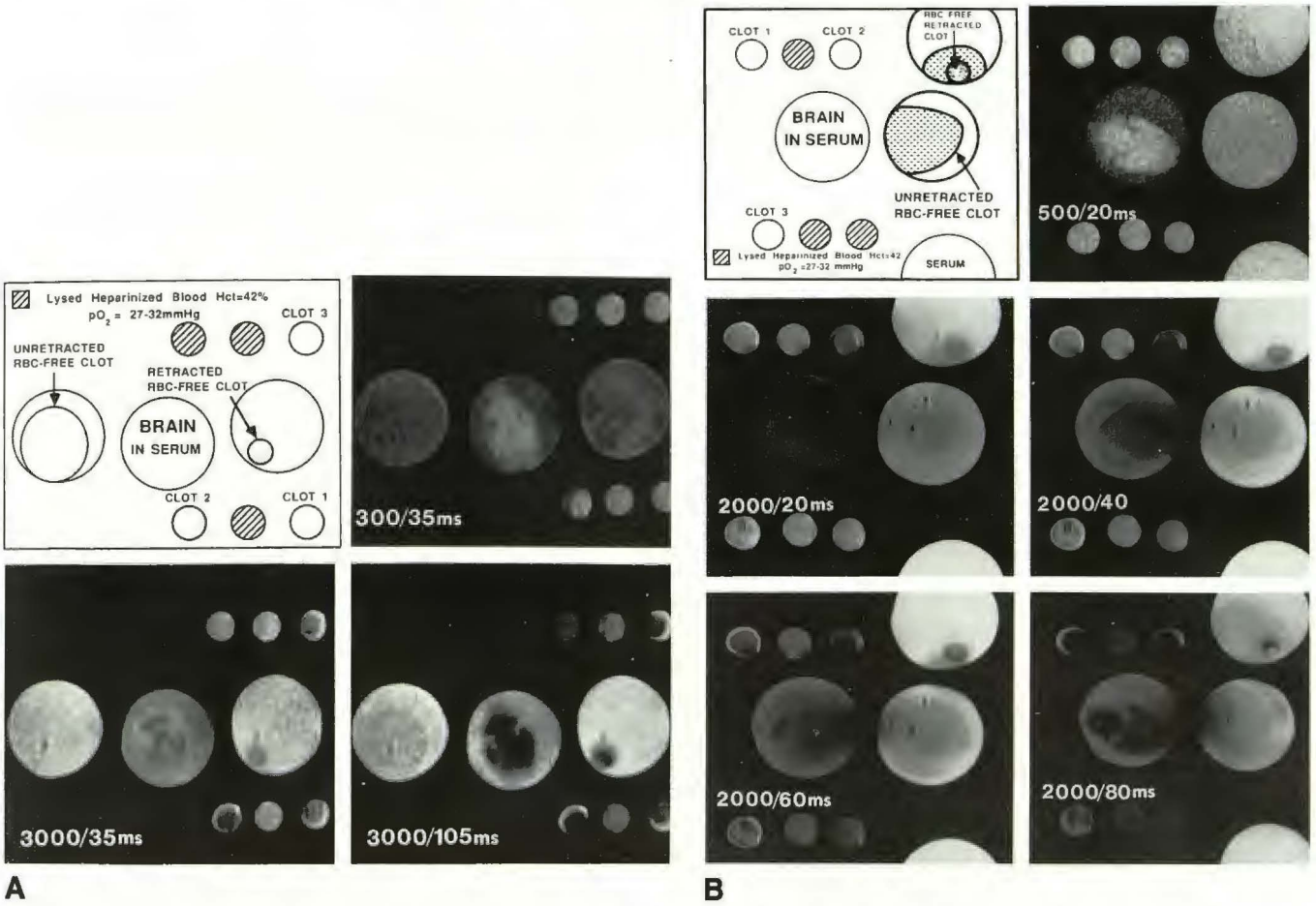

Fig. 2.—Phantom II. 0.5-T (*A*) and 1.5-T (*B*) spin-echo scans at TR/TE parameters shown. The key indicates the arrangement of samples within phantom. Note similar signal intensities in venous whole blood clots (clots 1, 2, and 3; pO_2 = 27–32 mm Hg) and the RBC-free retracted clot on the T2 and spin-density scan sequences. The companion 1.0-T images had an identical appearance.

Discussion

The mechanism of selective enhancement of T2 relaxation proposed by Gomori et al. [2] to account for the MR appearance of hemorrhage has received widespread attention in the radiology literature. This mechanism requires the intracellular presence of a paramagnetic agent. The data in the present study do not support this theory. Serial reduction in the signal intensity of a retracting clot that contained no RBCs was observed on T2-weighted spin-echo scans (Fig. 1). The decrease in signal intensity of RBC-free clots was considerably greater on late-echo (T2-weighted) as compared with early-echo (SD-weighted) images at all field strengths (Figs. 2 and 3), indicating that the majority of signal loss was due to an actual decrease in the T2 relaxation time, rather than to a decrease in SD.

Another line of evidence gathered in unclotted blood also indicates that the proposed selective enhancement of T2 relaxation is not a significant factor. A purely linear relationship has been shown to exist between hematocrit and the T2 relaxation time of blood, even at 4.7 T [3, 4]. The selective T2 theory predicts that a nonlinearity will occur when approx-

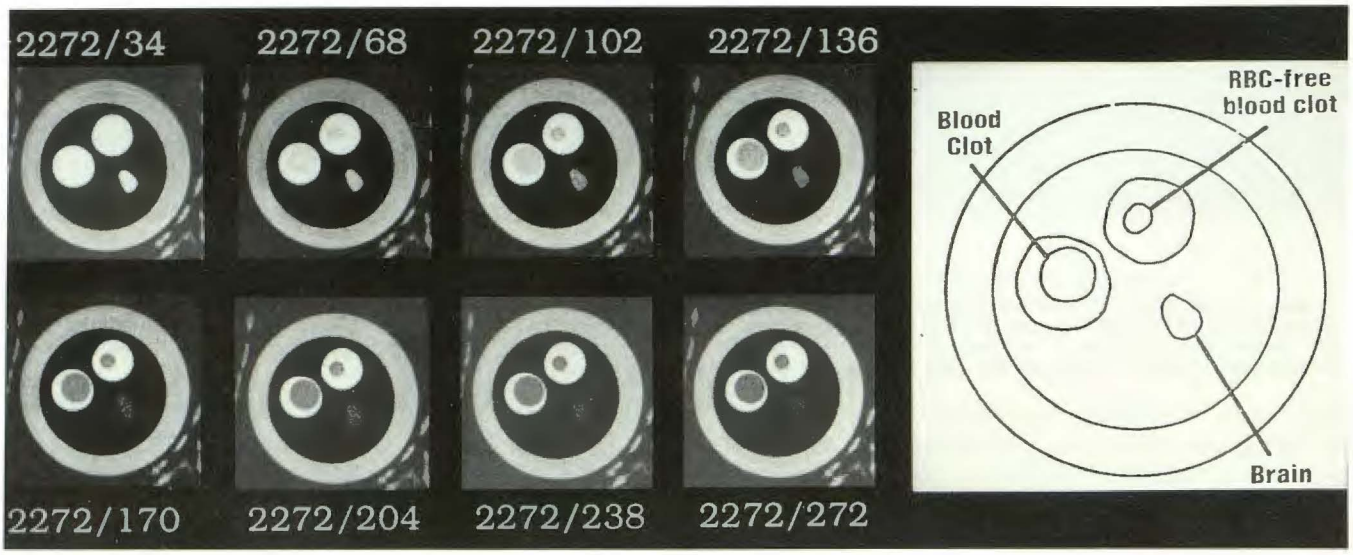

Fig. 3.—Phantom III. 0.3-T MR spin-echo scans at TR/TE parameters indicated. All images were photographed at identical window settings to show parallel signal intensities of retracted clot with and without intact red blood cells. On region of interest measurements the RBC-free clot and whole venous blood clot had almost identical values, but the brain sample had slightly greater signal intensity. The latter is difficult to appreciate visually because the brain is not surrounded by the bright signal of the plasma.

imately equal volumes of RBCs and plasma are analyzed [5]. The absence of the predicted nonlinearity indicates that the T2 shortening effect of increasing hematocrit is due purely to concentrating the RBCs.

In addition, no difference was noted in the signal intensity of hematoma formed from oxygenated or deoxygenated blood on T2-weighted spin-echo scans obtained at 1.5 T by using an in vivo cat model [6].

The observed reduction in signal intensity from both venous and RBC-free clots on T2-weighted spin-echo scans can most reasonably be attributed to the decrease in T2 relaxation that results from the increase in protein concentration and the decrease in free water concomitant with clot retraction [7]. During retraction the platelets and proteinaceous clotting factors are dramatically concentrated within the clot. The venous clot occupies a greater volume than the RBC-free clot, so the concentration of these factors will be lower in the venous clot. However, this will be offset by the high protein content within the RBCs. The net result is that both types of clots have comparable T2s and SDs. The venous clot has a shorter T1 relaxation time than RBC-free clot due to the T1 shortening effect of increased hematocrit [7]. Thus, the RBC-free clot is hypointense relative to both the brain and the venous clot on short TR, short TE (T1-weighted) spin-echo scans at 0.5 T, 1.0 T, and 1.5 T.

In the present study, none of the preparations recreated the clinical MR pattern in which hematoma is hypointense relative to brain. Clearly, another, yet to be identified, factor or factors must further shorten the T2 of hematoma to reduce its signal intensity below that of brain.

In summary, clots that contain no red blood cells have very similar signal intensities compared with clots containing RBCs on T2-weighted images at field strengths of 0.3–1.5 T. The T2 shortening effect of clot retraction appears as clot formation commences and is attributed to the increase in protein concentration. In normal blood it should probably be observed within at least 4 hr after extravasation of normal blood into the extravascular space. Patients with abnormal platelets or bleeding disorders, which allow continued extravasation of fresh blood, will have hemorrhages in which part or all of the lesion does not have the reduction in T2 signal intensity caused by retracted clot formation and the increased hematocrit it creates. The influence of the subsequent dissolution of clot matrix on MR signal intensities remains to be explored. Additional factor(s) must be present to reduce the signal intensity of clot below the levels of normal brain.

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REFERENCES

1. Hayman LA, Pagani JJ, Kirkpatrick JB, Hinck VC. Pathophysiology of acute intracerebral and subarachnoid hemorrhage: applications to MR imaging. *AJNR* **1989**;10:457–461, *AJR* **1989**;153:135–139
2. 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
3. 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
4. Fullerton GD, Potter JL, Dornbluth NC. NMR relaxation of protons in tissues and other macromolecular water solutions. *Magn Reson Imaging* **1982**;1:209–228
5. Thulborn KR, Waterton JC, Matthews PM, Radda GK. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochem Biophys Acta* **1982**;714:265–270
6. Hayman LA, McArdle CB, Taber KH, et al. MR of hyperacute intracranial hemorrhage in the cat. *AJNR* **1989**;10:681–686
7. Linstrom TR, Koenig SH. Magnetic field dependent water protein spin lattice relaxation rates of hemoglobin solution and whole blood. *J Magn Reson* **1974**;15:344–352