Subarachnoid hemorrhage has a much higher intensity in magnetic resonance (MR) images with the passage of time. Acute subarachnoid hemorrhage is difficult to see; within 1 week its appearance has become intensified on T1-weighted images. Different concentrations of blood and lysed red blood cells in cerebrospinal fluid (CSF) were examined spectroscopically but did not significantly alter T1 and T2 relaxation of CSF acutely. Ultraviolet visible spectroscopy of bloody CSF stored hypoxically for 3 days showed the presence of methemoglobin. The iron in methemoglobin is paramagnetic; in combination with water this facilitates T1 relaxation. It is concluded that methemoglobin formation with T1 shortening at least partially accounts for the increasing intensity of the MR appearance of subarachnoid hemorrhage over time in the central nervous system and may also explain the intense appearance of subacute hemorrhage in MR images elsewhere in the body.

Index terms: Brain, hemorrhage • Hemorrhage, magnetic resonance studies • Red blood cells

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Effect of Methemoglobin Formation on the MR Appearance of Subarachnoid Hemorrhage

The earliest reports of magnetic resonance (MR) images of intracranial hemorrhage (1, 2) suggested a relatively intense appearance of the hemorrhage relative to the surrounding brain. This was attributed to the short T1 relaxation time of the presumably paramagnetic, iron-containing hemoglobin. Figure 1 illustrates this appearance in a patient imaged 1 week after rupture of an aneurysm in an anterior communicating artery, with resulting intraparenchymal hematoma and subarachnoid hemorrhage. The short T1 character of the injured area is enhanced relative to surrounding brain on a T1-weighted spin-echo image.

As experience was gained, subsequent reports (3, 4) indicated that acute intracranial hemorrhage could be much more difficult to detect on MR images. This was attributed by Sipponen et al. (3) to a lack of T1 shortening during the acute phase; however, they attempted no explanation of this phenomenon. They reported that acute subarachnoid hemorrhage was difficult to detect by MR study, particularly in comparison with computed tomography (CT) (4). Although DeLaPaz et al. (4) agree that acute intracranial hemorrhage is difficult to detect on MR images, they disagree as to the mechanism causing the intense appearance. While the data of Sipponen et al. (3), Bailes et al. (1), and Bydder et al. (2) suggest a T1 shortening process, the data of DeLaPaz et al. (4) suggest no change in T1 but rather a prolongation of T2, both of which would increase the intensity on spin-echo images. Figure 2 illustrates the rather subtle increased intensity present in acute subarachnoid hemorrhage 17 hours after ictus. One week after the acute subarachnoid hemorrhage, the appearance is significantly more intense (Fig. 3).

To attempt an understanding of the variable appearance of subarachnoid hemorrhage on MR images, the structure of hemoglobin and its various breakdown products must be considered in some detail. In its circulating form, hemoglobin alternates between the oxy and deoxy forms as oxygen is exchanged during its transit through the high-oxygen environment of the lungs and low-oxygen environment of the capillary circulation. To bind oxygen reversibly, the iron in the hemoglobin (heme iron) must be maintained in the reduced ferrous (Fe²⁺) state (5). To do this, the red blood cell maintains several metabolic pathways to prevent various oxidizing agents from converting its heme iron to the nonfunctional ferric (Fe³⁺) state. When hemoglobin is removed from the circulation, these metabolic pathways fail and the hemoglobin molecule begins to undergo oxidative denaturation.

The heme iron normally is suspended in a nonpolar crevice in the center of the hemoglobin molecule. It is held in this position by a covalent bond with a histidine at the so-called F8 position of the globin chain and by four planar hydrophobic van der Waals bonds with various nonpolar groups on the globin molecule. The group attached to the sixth coordination site of the heme iron varies. It is occupied by molecular oxygen in oxyhemoglobin; it is vacant in deoxyhemoglobin (Fig. 4). As the oxidative denaturation of hemo-
globin proceeds, the ferrous heme iron is oxidized to the ferric state and methemoglobin is formed. The five bonds to the globin molecule are unchanged; the sixth coordination site is now occupied by either a water molecule or a hydroxyl ion, depending on whether the methemoglobin is acidic or basic. At physiologic pH, the acid form predominates. With continued oxidant denaturation, methemoglobin is converted to derivatives known as hemicromes (5). While the iron in these compounds remains in the ferric state, alteration of the tertiary structure of the globin molecule occurs, with the result that the sixth coordination site of the heme iron is occupied by a ligand from within the globin molecule (most likely the distal histidine at the E7 position).

To consider the effect of subarachnoidal hemorrhage on MR imaging over time, one must evaluate the interaction between the iron-containing hemoglobin within the red blood cells and water protons in the cerebrospinal fluid (CSF). A trivial explanation for the T1 shortening apparent on images of subacute hemorrhage might be the lysis of red blood cells that can occur as a result of their exposure to phospholipases in the CSF. We can readily exclude this mechanism for enhanced proton relaxation because water molecules are already allowed access to the hemoglobin by rapid transit across the red cell membrane (6, 7). Thus, changes in proton relaxation enhancement effects may be attributed directly to changes in the interactions between the CSF water protons and the heme iron that occur as a result of oxidative denaturation of hemoglobin.

In view of the discrepant reports in the MR imaging literature and the lack of an obvious explanation from the literature on MR and ultraviolet-visible (UV) spectroscopy for the changing appearance of subarachnoidal hemorrhage, the following studies were undertaken.

**MATERIALS AND METHODS**

Subarachnoidal hemorrhage was modeled in vitro by adding fresh venous human blood to artificial CSF, producing a 10% (by volume) solution. The T1 and T2 relaxation times of this solution were evaluated using an IBM Minispec desktop spectrometer operating at 20 MHz. The effect of red blood cell concentration on T1 and T2 relaxation was evaluated by measuring the relaxation times at concentrations varying from 0% (pure CSF) to 10% by volume. The effect of red blood cell lysis was evaluated by mechanically lysing the red blood cells; to do this, fresh venous blood was repeatedly passed through a 25-gauge needle before being mixed with CSF. The magnetic relaxation times of oxy- and deoxyhemoglobin were compared by bubbling either oxygen or nitrogen through fresh solutions of bloody CSF. Methemoglobin was produced by treatment of deoxyhemoglobin with sodium nitrite (NaNO2). Before measurement, all samples were agitated to suspend the red cells. All measurements were performed at 38°C.

Quantitation of subarachnoid hemorrhage generally is performed by means of light (visible) spectroscopy (8). Such analysis provides the "xanthochromic" index that is used to quantitate the degree of hemorrhage. The xanthochromic index is the sum of the absorption values at 415 nm (oxyhemoglobin) and 460 nm (bilirubin). All samples are prepared for spectrophotometric analysis by centrifugation, with examination of only the supernatant. By such analysis, little methemoglobin has previously been found either in acute subarachnoid hemorrhage or within several weeks after the hemorrhage (8, 9).

The solutions of oxy- and deoxyhemoglobin and methemoglobin were analyzed using a Perkin-Elmer UV-visible spectrophotometer. Attention was directed to the 630-nm region, which is specific for methemoglobin.

To assess the effect of oxy- and deoxyhemoglobin on the "unknown" (bloody CSF) and compared with the known standards previously produced.

**RESULTS**

The effect of concentration of blood in CSF is demonstrated in Figure 5, which shows a small (10%) decrease in T1 and T2 relaxation times as the concentration is increased from 0% (pure CSF) to 10% red blood cells. Such T1 shortening clearly is not the primary mechanism for the increased intensity observed in subarachnoidal hemorrhage clinically. Figure 5 also

![Figure 1](image1.png)

**Figure 1**

Subacute subarachnoid hemorrhage. Intraparenchymal hematoma (arrow) and subarachnoid hemorrhage (arrowhead) are noted 1 week after rupture of aneurysm in anterior communicating artery. The contrast between the lesions and the surrounding brain is enhanced on this T1-weighted spin-echo image (TR = 0.5 sec; TE = 28 msec).

![Figure 2](image2.png)

**Figure 2**

A. Acute subarachnoid hemorrhage 17 hours post ictus.
B. Midline sagittal section shows minimally increased intensity in the pontine and medullary cisterns (arrowheads). The intensity of the CSF in these subarachnoid spaces should be the same as that in the fourth ventricle (arrow).

B. Coronal section demonstrates minimally increased intensity in the left sylvian cistern on this TR 1.5-sec, TE 28-msec image.

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Subacute subarachnoid hemorrhage 1 week post ictus. Intensity of the CSF in the left sylvian cistern is significantly greater than observed during the first 24 hours post ictus. (TR = 1.5 sec, TE = 28 msec.)

Change in magnetic relaxation times (20 MHz) as a function of red blood cell concentration in CSF and red blood cell lysis. A 10% decrease in the T1 (above) and T2 (below) times is noted as a function of increasing concentration of red blood cells in CSF from pure CSF to 10% by volume. There is no significant effect of red blood cell lysis on T1 or T2 relaxation.

Demonstrates that the effect of red blood cell lysis is negligible.

As shown in Figure 6, T1 measurements of oxy- and deoxyhemoglobin are quite similar, confirming what has been reported previously (10). Methemoglobin in a 10% solution of lysed red blood cells in CSF is seen to have a significantly lower T1. No significant difference in T2 was found among oxy-, deoxy-, and methemoglobin. Figure 6 also illustrates the sequential decrease in T1 for the 10% "unknown" solution of lysed red blood cells in CSF over 84 hours of measurement. Figure 7 shows T1 shortening of a 20% solution of whole red blood cells in CSF stored hypoxically for 160 hours. The T1 value continues to shorten for 90 hours and then plateaus.

Spectrophotometry of both unknown solutions of bloody CSF demonstrate strong absorption at 360 nm, called the Soret band (Fig. 8). Strong absorption is present for all forms of hemoglobin in this region; thus it is not specific. That portion of the spectrum more specific for methemoglobin is the 630-nm region, which is shown in Figure 9. Here temporal increase in a broad peak is observed in the "unknown" 20% bloody CSF solution, which corresponds to increasing methemoglobin concentration. Although there is significant difference in the height of the 630- and 360-nm peaks, this reflects differences in the extinction coefficients rather than differences in concentration. Thus, by comparison with known standards, the peak observed at 92 hours at 631 nm is shown to correspond approximately to 90% methemoglobin.

The magnetic properties of dried blood were first evaluated by Faraday 140 years ago (10); 90 years later Pauling and Coryell (11) considered the magnetic properties of blood in the fluid state. By using a capillary tube filled with either oxy- or deoxyhemoglobin suspended between the poles of an electromagnet, they were able to determine that deoxyhemoglobin was paramagnetic (attracted to the stronger part of the magnetic field), while oxyhemoglobin was diamagnetic (repelled from the stronger part of the magnetic field). These observations led them to describe the various electron spin states of oxy- and deoxyhemoglobin. In deoxyhemoglobin, the heme iron is in the "high spin" ferrous state characterized by six electrons in the outer (d) shell, four of which are unpaired. When oxygen is added, one of the electrons is partially transferred to the oxygen molecule, resulting in a low spin form with a single unpaired electron in the outer shell (5).

Although the static susceptibility test performed by Pauling and Coryell demonstrates that deoxyhemoglobin is paramagnetic, this does not ensure a proton paramagnetic enhancement effect in aqueous solution. Such an effect was originally described by Bloembergen et al. (6) and
requires not only that a paramagnetic center be present but also that it be accessible to surrounding water protons. The quantitation of this effect requires consideration both of the magnitude of the magnetic moment of the paramagnetic dipole (the number of unpaired electrons), the electron spin relaxation rate (in effect, the T1 of the electron), the concentration of paramagnetic dipoles, the average distance from surrounding water protons, and the relative motion of the proton and paramagnetic centers. Such theories of proton relaxation by paramagnetic solute ions are based on translational diffusion and the distance of closest approach of the proton and paramagnetic ions, which determines an "outer sphere" of influence (12). It has also been shown that there can be a contribution to relaxation from exchange between solvent and water ligands in the first coordination sphere of the paramagnetic ion, that is, "inner sphere effects" (12). Thus, while the deoxy form of hemoglobin is considered paramagnetic on the basis of static susceptibility experiments (11), the T1 relaxation times of aqueous solutions of oxy- and deoxyhemoglobin do not show a difference in proton paramagnetic relaxation effects (13). Methemoglobin, on the other hand, causes significant T1 shortening in aqueous solution due to a combination of both "inner sphere" and "outer sphere" effects (12). Thus T1 relaxation by methemoglobin is due to a combination of ligand-exchange effects (from the water molecule at the sixth coordination site) and from outer sphere diffusional effects, perhaps because solvent protons have increased access to the heme iron through the nonpolar crevice (12).

T1 shortening has been demonstrated during hypoxic storage of two solutions of red blood cells in CSF that were intended to simulate subarachnoid hemorrhage. The same T1 shortening has been observed clinically, as shown in Figures 1–3. In contrast to DeLaPaz et al. (4), we did not find a prolongation of T2.

Sequential spectrophotometric analysis of the solution shows a corresponding increase in the concentration of methemoglobin during the period of T1 shortening. Because oxy- and deoxyhemoglobin are known to have an insignificant effect on proton relaxation enhancement in aqueous solutions (6), we presume that methemoglobin is responsible for both the T1 shortening observed in the current experiments and that seen clinically. Furthermore, we believe that methemoglobin may be responsible for the intense, short T1 appearance of subacute or chronic hemorrhage observed elsewhere in the brain and the rest of the body.

Although we believe this is compelling evidence, it should be emphasized that the experiment described here may differ somewhat from the in vivo clinical situation. Various enzyme systems may affect the subarachnoid space that are not present here (14). In addition, red blood cells may be cleared from the subarachnoid space more rapidly (15) than is required to produce T1 shortening by methemoglobin formation. Thus, extension of this explanation to the clinical situation, while strongly suggested, has not been fully verified. We have detected methemoglobin in a subacute subdural hematoma and believe it to account (at least partially) for the T1-shortening observed clinically. Spectrophotometric and MR analysis of clinical subarachnoid hemorrhage are in progress.

The apparent disagreement between our findings and previous spectrophotometric studies that demonstrated minimal methemoglobin might be resolved by comparison of the differing methods of sample preparation. In the reported spectrophotometric analyses, particulate scattering causes significant degradation, necessitating centrifugation. With MR, such centrifugation is not necessary and in fact results in a significant prolongation of the measured T1 values. Thus, it would appear that the paramagnetic methemoglobin is either held within the intact red blood cell or is adsorbed to the lysed red blood cell membrane, both of which will be carried to the bottom of the tube during centrifugation and not be present in the supernatant to cause significant absorption during UV spectroscopy.

Regardless of the exact mechanism of T1 shortening over the first week following subarachnoid hemorrhage, it should be emphasized that the appearance of acute subarachnoid hemorrhage is much less striking on MR images than on CT scans. For this reason, we fully agree with DeLaPaz et al. (4) that CT is more sensitive and specific in this evaluation. CT should thus be considered the examination of
Spectrophotometry near 600 nm. Oxyhemoglobin is seen to have no absorption at 630 nm. During hypoxic storage of a solution of blood and CSF, a broad-based peak develops at 630 nm. By comparison with known standards, the peak at 92 hours was shown to correspond to 90% methemoglobin. Differences in peak heights reflect differences in extinction coefficients.

choice for evaluating patients following an acute intracerebral event that could include hemorrhage. However, patients evaluated in the subacute stage (1 week or more post ictus) may be better studied by MR imaging as, by that time, T1 shortening will occur, allowing a specific diagnosis of hemorrhage.

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Send correspondence and reprint requests to: William G. Bradley, Jr., M.D., Ph.D., Huntington Medical Research Institutes, MR Imaging Laboratory, 10 Pico Street, Pasadena, CA 91105.

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