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AJNR Am J Neuroradiol 1989, 10 (4) 681-686
http://www.ajnr.org/content/10/4/681

MR Imaging of Hyperacute Intracranial Hemorrhage in the Cat

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Hyperacute intracerebral hematomas were successfully created in five cats by injecting a prepared blood sample in which the oxygen (O_2) saturation ranged from 0–80%. T1- and T2-weighted spin-echo sequences and T2-weighted gradient refocused scans were obtained 2.5–10 hr after injection on a 1.5-T imaging system. Detailed histology or electron microscopy was performed on each brain to confirm the presence of intact red blood cells in a retracted clot matrix. Areas of the hematoma were hypointense relative to brain in all five cats on the gradient refocused scans. The hematoma was isointense relative to brain on the T1- and T2-weighted spin-echo scans in all cats except one, which suffered a seizure/respiratory arrest and died during the scanning procedure. Portions of the hematoma in this animal had a hypointense T2-weighted signal and a hyperintense T1-weighted signal, which corresponded to the predicted MR properties of intracellular methemoglobin.

We hypothesize that acute (<10 hr old) hematomas that contain virtually 100% intracellular deoxyhemoglobin may not appear hypointense relative to brain on T2-weighted scan sequences at 1.5 T unless surrounding tissue hypoxia and/or anoxia promote additional changes, one of which may be the formation of intracellular methemoglobin.

It has been postulated that an increase in the concentration of intracellular deoxyhemoglobin (Hb) and/or methemoglobin (MHb) is responsible for the MR imaging characteristics of acute intracerebral hemorrhage [1]. However, the oxygenation state of the hemoglobin in acute intracerebral hematoma has not been measured in vivo. To circumvent this difficulty, intracerebral hematomas were created in five cats by using blood with stable concentrations of Hb and MHb. The ability of spin-echo (SE), T1-, and T2-weighted pulse sequences to detect and characterize these lesions was compared with that of T2-weighted gradient-refocused images obtained at 1.5 T.

Materials and Methods

Five adult male cats weighing 3–3.5 kg were anesthetized. The first three cats were given 0.5 ml of ketamine hydrochloride (100 mg/ml)* intramuscularly; cats 4 and 5 were given chloralose† intravenously. All cats received supplemental halothane anesthesia prior to surgery. A scalp incision was made to expose the skull, and a left frontal burr hole was drilled. Three milliliters of venous blood were taken from the cats' femoral vein and deoxygenated with a few grains of sodium hydrosulfite (Fisher). An 18-gauge needle was inserted ¼ in. into the brain and 2–3 ml of the blood was injected at a rate of 0.5 ml/min. In the first three cats, a Harvard Apparatus Pump, Model 940‡ was used. In the last two cats a timed injection was made by hand to approximate the 0.5 ml/min rate. The remaining 0.5 ml sample of blood was immediately analyzed. A CO-oximeter§ was used for measuring the % saturation of oxyhemoglobin (O_2Hb) and MHb concentration in cats 1–3. A Corning blood gas analyzer was used for measuring the pO_2 in cats 4 and 5. No MHb measurement was done in cats 4 and 5. The amount of Hb was estimated by totaling the O_2Hb and MHb (when available) and subtracting from 100.

Received January 13, 1988; revision requested March 14, 1988; revision received December 11, 1988; accepted December 14, 1988.

This work was supported by NIH grant NS19056.

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AJNR **10:681–686, July/August 1989**
0195–6108/89/1004–0681

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The MR scanning was performed in the extremity or head coil on a 1.5-T GE unit. A multislice sequence with a 5-mm slice thickness and 1.5-mm gap was used with four excitations and a 256 × 128 matrix. The scan sequences used for each cat are listed in Table 1. Gradient-refocused (GR) sequences were performed with a partial saturation interleaved (PSI) data base in cats 1–3 and with a gradient-recalled acquisition in the steady state (GRASS) in cats 4 and 5. Flip angles of 30° were used for PSI and 10° for GRASS. The times between the injection of blood and the beginning of the MR scanning session or animal sacrifice are shown in Figure 1. The MR signal intensities observed in the hematoma with each scan sequence were characterized as hypointense, isointense, or hyperintense relative to the unoperated hemisphere.

After the images were collected, a supplemental dose of ketamine anesthesia was administered to cats 1–4 and they were sacrificed by decapitation at the times shown in Figure 1. Cat 5 had a respiratory arrest and died during MR scanning. After death, the brains of all the cats were immediately removed. The brains of cats 1 and 2 were immediately frozen. Within 3 days they were thawed, sectioned, and visually compared with the MR scans. These clots were then removed and fixed in glutaraldehyde. Electron microscopic (EM) sections were prepared from these samples and from cat blood, which was allowed to stand without anticoagulants for 15 min. The fibrin strand density on EM was assessed to judge the degree of clot retraction present in the cat brain hematomas as compared with the unretracted peripheral blood clot.

The brains of cats 3–5 were fixed in formalin for 2 weeks and sectioned in the coronal plane. Large-mount histology sections were obtained and stained with hematoxylin and eosin (H and E). The size of the hematoma was visually compared with each of the MR scans done at the same coronal level in cats 3–5.

To test the stability of the deoxygenated blood preparation, a separate in vitro experiment was performed. Venous blood, drawn from a volunteer donor, was reduced by the addition of sodium hydrosulfite (Fisher). The samples (n = 3) were maintained at 37°C under nitrogen for 29 hr. Aliquots were analyzed for % saturation of O_2Hb and MHb at 0, 6, 19, and 28 hr on a CO-oximeter.

Results

Cats 1–4 survived the surgery and MR scanning procedures. Cat 5 had a seizure immediately after the intracerebral injection of blood, suffered a respiratory arrest, and was resuscitated and maintained by assisted ventilation. He died during MR imaging.

TABLE 1: Signal Intensity of the Intracerebral Hematoma Compared with Normal Brain

	Cat No.				
	1	2	3	4	5
(% O_2 saturation)[a]	(59%)	(73%)	(80%)	(0%)	(0%)
SE 2500/80	0	0	0	0	—
SE 2500/160	0	0	0	ND	ND
SE 4000/80	0	0	0	ND	ND
SE 4000/160	0	0	0	0	−
SE 500/20	0	0	0	0	+, 0
SE 4000/35	ND	ND	ND	+, 0	ND
PSI 750/10,40[b]	−	−	−	ND	ND
GR 750/40[b]	−	−	−	−	−, +

Note.—+ = hyperintense, 0 = isointense, − = hypointense, ND = not done.

[a] Measured pO_2 values of 0 were converted to % saturation for consistency within this table.

[b] PSI flip angle = 30° for cats 1–3, GRASS flip angle = 10° for cats 4 and 5.

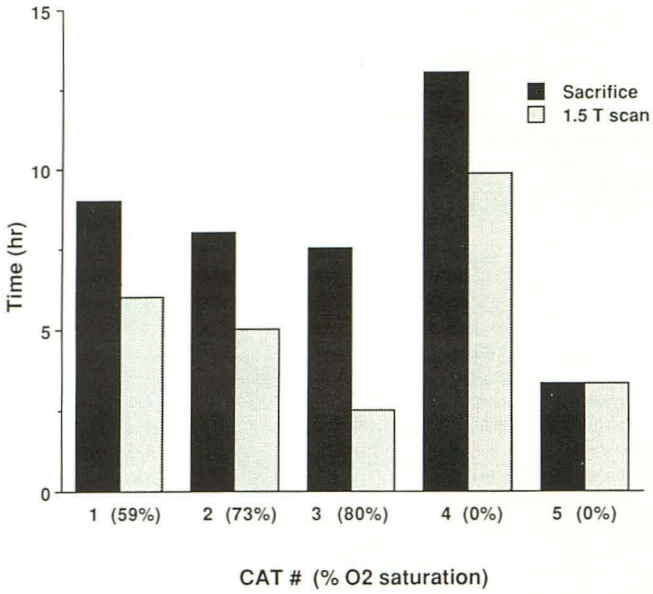


Fig. 1.—MR scan/sacrifice protocol. Blood was injected into the brain at time 0. MR scan sessions began 2.5 to 10 hr after injection. Cat 5 died during the scan. Cats 1–4 were sacrificed 9–12.5 hr after injection.

The presence of an intracerebral hematoma was confirmed by visual inspection of the sectioned brains in all five cats. The size of the lesions indicated that only a small portion of the 2–3 ml of blood injected into the brain remained in the hematoma. Most of the blood flowed out of the needle track into the subarachnoid, subdural, and extradural spaces. A large subarachnoid blood clot was present on the brain surface of cat 5 when the skull was opened. None of the MR scans detected this lesion. The small size of the intracerebral hematomas precluded analysis of the chemical state of the hemoglobin molecules.

The image intensity of intracerebral hematoma compared with normal brain is summarized in Table 1. Figures 2–4 illustrate the histologic and MR findings. Electron microscopy demonstrated numerous zones of tightly packed fibrin strands compared with the unretracted clot found in the cats' peripheral blood sample. This confirmed the presence in cats 1 and 2 of retracted clot formation in both of the hematomas removed 8–9 hr after injection.

The gradient echo was the most sensitive scan sequence. It demonstrated an area of low signal intensity in cats 1–4 and a mixture of hyperintense and hypointense signal in cat 5. The size of the hematoma was exaggerated on late-echo images compared with the early-echo images with a flip angle of 30° in cats 1–3 (see Fig. 2).

The T2-weighted scans failed to demonstrate an area of hypointense signal in cats 1–4, even though the blood in cat 4 was devoid of O_2Hb. A portion of the hypointense signal on the T2-weighted images from the blood in cat 5 was accompanied by a corresponding zone of increased signal on the

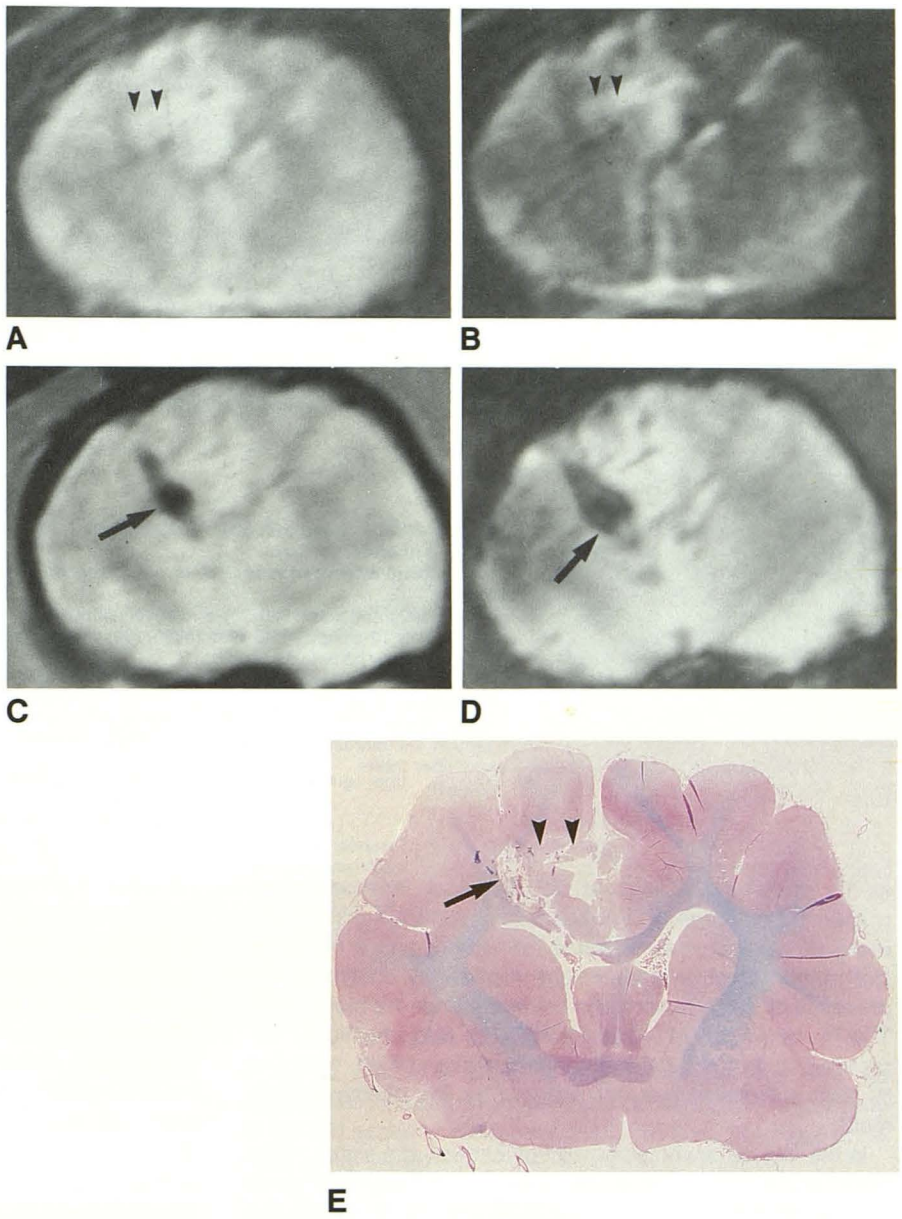


Fig. 2.—Cat 3. Blood with an 80% O_2 saturation was injected. The cat survived the surgery and scan procedures, which began 2.5 hr after injection.

***A* and *B*, T2-weighted scans at 4000/80 (*A*) and 4000/160 (*B*) show hematoma to be isointense relative to brain. Brain edema is shown as hyperintense zone medial to hematoma site (*arrowheads*).**

***C* and *D*, Gradient-refocused scans at 750/10 (*C*) and 750/40 (*D*) with a 30° flip angle show hypointense signal in region of hemorrhage (*arrows*). Note that region is artifactually enlarged on the 750/40 scan. This is caused by blooming effect, which occurs on late-echo scan sequences.**

***E*, H and E stain of corresponding coronal brain slice shows intracerebral hemorrhage (*arrow*) at 8 hr after injection. At microscopy, blood cells of hematoma were intact and edema was present in fragmented brain tissue medial to hemorrhage (*arrowheads*).**

T1-weighted images within the hematoma. This pattern was not seen in cats 1–4.

The percent oxygen saturation of the blood injected into each cat brain is shown in Table 1. The measured % MHb concentrations in cats 1–3 were within the normal range (1–5%). The in vitro study directly verified the stability of the O_2Hb and MHb levels in the blood preparations and, indirectly, the Hb blood levels over 28 hr. At time zero, virtually all the hemoglobin was in the form of Hb (99.6%, 99.1%, and 94.4% saturation). At 6 hr there was little change in the Hb levels (98.3%, 97.9%, 95.7% saturation). By 19 and 28 hr there was a slight decrease in the Hb levels (86.1%, 95.7%, 93.4% saturation) and (87.8%, 83.1%, 96.5% saturation), respectively. The MHb levels remained in the normal range in all three samples throughout the study. Therefore, under the inert conditions presumably present in the viable animals (cats 1–4) the percent Hb would appear to remain unchanged between the time of injection and the scan. In other words, spontaneous oxygenation of the sample or an increase in MHb do not occur in this chemically treated blood unless other factors intervene.

Discussion

Several factors are known to decrease the T2 relaxation time of blood. These factors are (1) the presence of the intracellular paramagnetic agents deoxyhemoglobin (Hb) and methemoglobin (MHb) [2], (2) the formation of a retracted clot matrix [3], and (3) an increase in hematocrit [4]. The present

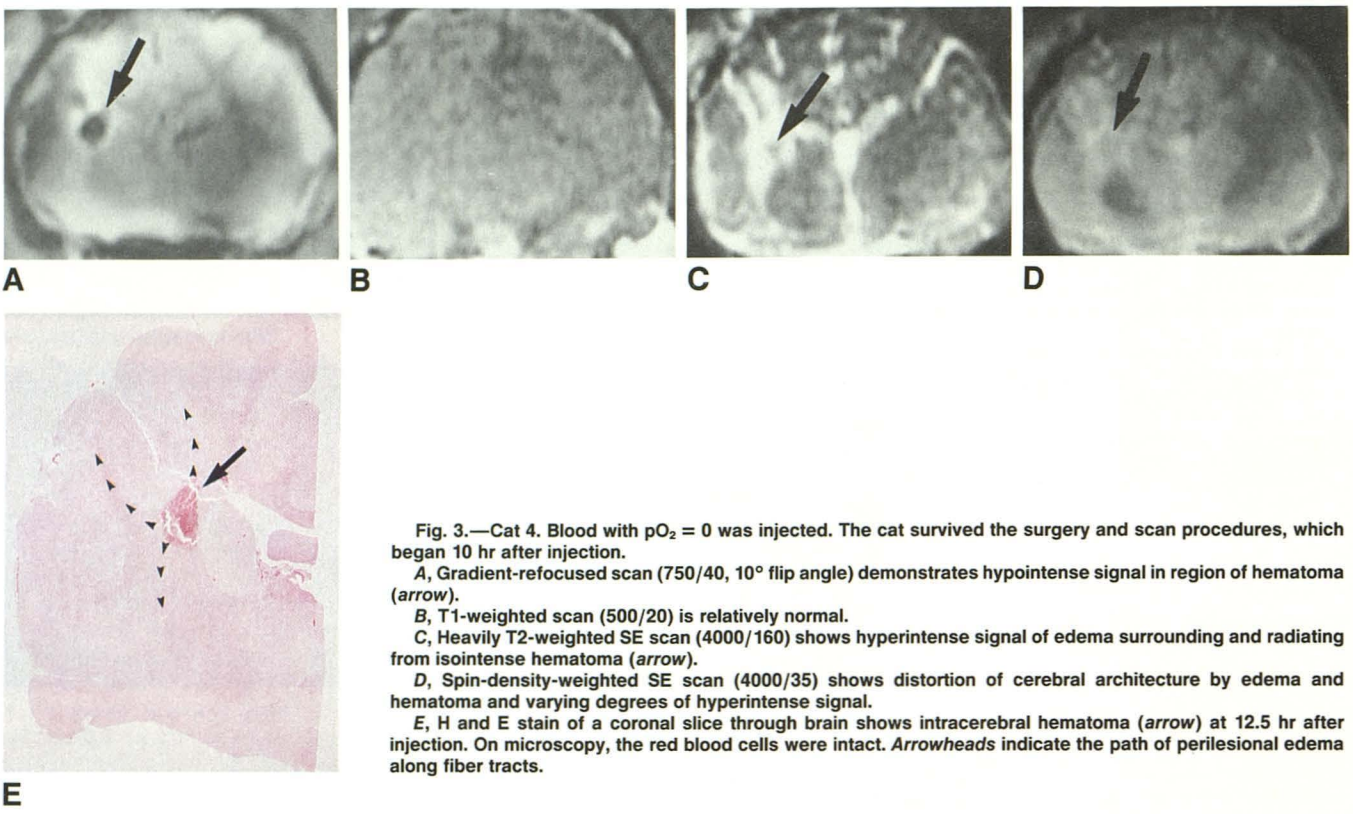


Fig. 3.—Cat 4. Blood with $pO_2 = 0$ was injected. The cat survived the surgery and scan procedures, which began 10 hr after injection.

***A*, Gradient-refocused scan (750/40, 10° flip angle) demonstrates hypointense signal in region of hematoma (*arrow*).**

***B*, T1-weighted scan (500/20) is relatively normal.**

***C*, Heavily T2-weighted SE scan (4000/160) shows hyperintense signal of edema surrounding and radiating from isointense hematoma (*arrow*).**

***D*, Spin-density-weighted SE scan (4000/35) shows distortion of cerebral architecture by edema and hematoma and varying degrees of hyperintense signal.**

***E*, H and E stain of a coronal slice through brain shows intracerebral hematoma (*arrow*) at 12.5 hr after injection. On microscopy, the red blood cells were intact. *Arrowheads* indicate the path of perilesional edema along fiber tracts.**

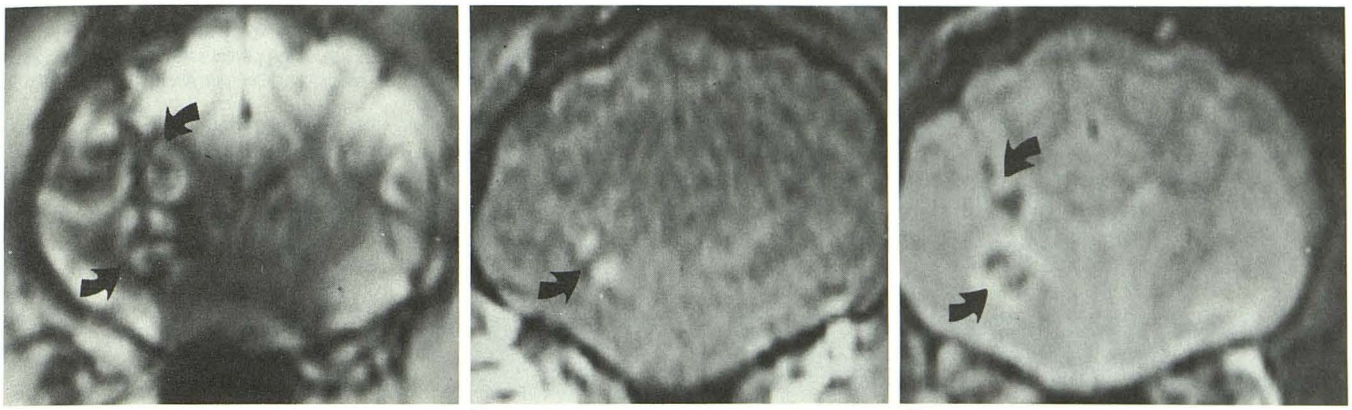
study utilized blood without anticoagulant to produce retracted intracerebral clots that would maximize the T2 shortening properties of an elevated hematocrit and a retracted clot matrix. Electron and/or light microscopy confirmed the presence of these factors and the integrity of the blood cells. The % saturation of intracellular O_2Hb was chemically decreased by the formation of varying degrees of Hb, while the % MHb saturation was not affected by this chemical treatment.

In the four cats who survived the surgery, the signal of the hematoma on T2-weighted SE scans at 1.5 T was isointense relative to brain. This group included a cat in whom the injected blood had a pO_2 of zero and the clot was allowed to retract over 10 hr before the MR scans were started (Fig. 3). Since fully deoxygenated and clotted blood was isointense relative to brain, other as yet undiscovered factors must affect the T2 relaxation time of intracerebral blood collections sufficiently to result in a hypointense signal on clinical SE T2-weighted scans.

The fifth cat, who suffered a seizure, respiratory arrest, and died during the scan, offers some insight into the in vivo processes that may influence the MR signal intensity of hyperacute intracerebral blood. Severe tissue anoxia and/or death appears to trigger changes that result in a hypointense signal on SE T2-weighted images. Portions of this hypointensity could be the result of intracellular MHb formation. The presence of this agent would explain the hyperintense signal noted on T1-weighted images (Fig. 4). In phantom studies, we have noted that the pattern of hyperintense signal on T1-weighted images, combined with a hypointense signal on T2-weighted scans, is only found in blood samples and/or clots that contain intracellular methemoglobin [5].

If the rate of intracellular MHb formation is strongly influenced by the biochemical state of surrounding tissue, and if these levels can be detected on MR scans, this technique could be used to noninvasively monitor the presence of tissue hypoxia/anoxia. Subtle transient zones of increased signal on T1-weighted scans have been noted in acute MR scans of patients [6]. Additional studies are planned to investigate this possibility and to look for the other factors that can further decrease the T2 relaxation time of blood.

Many studies have indicated the ability of gradient-refocused MR scanning to detect blood that was not seen on SE sequences at low and high field strengths [7–9]. Gomori et al. [1] suggested these sequences are sensitive to the presence of magnetic susceptibility from intracellular deoxyhemoglobin. Weingarten et al. [10] showed that the gradient sequences at 0.6 and 1.5 T are sensitive in vivo to extremely low Hb concentrations by demonstrating a hypointense signal on gradient-echo scans in three dogs injected with arterial blood that did not contain an anticoagulant. In our study, the gradient-echo sequences showed zones of hypointense signal in the region of the hematoma in all five cats regardless of the Hb saturation in the sample. This group included a cat (no. 3) in which the red blood cells had a % oxygen saturation of 80 (i.e., a % Hb saturation of only 15). The biological basis



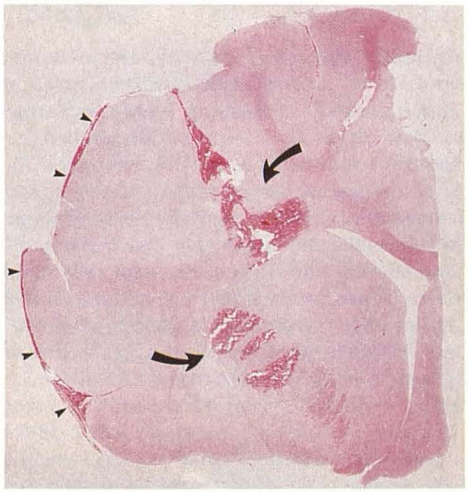


Fig. 4.—Cat 5. Blood with a $pO_2 = 0$ was injected. Immediately after injection, the cat suffered a grand mal seizure and respiratory arrest. Despite assisted ventilation, the cat died during MR scanning, which began 3.3 hr after injection.

***A*, Gradient refocused scan (750/40, 10° flip angle) shows a mixture of hyperintense and hypointense signal from both zones of hemorrhage (*arrows*).**

***B*, T1-weighted scan shows hyperintense signal in deepest zone of hemorrhage (*arrow*).**

***C*, T2-weighted SE scan (4000/160) shows hypointensity in both zones of hemorrhage (*arrows*).**

***D*, H and E stain of a coronal slice through hemisphere shows both zones of intracerebral hemorrhage (*arrows*) and subarachnoid blood (*arrowheads*). At microscopy, there was slight fragmentation of less than 1% of the red blood cells, but no ghost cells were present. The remainder of the red blood cells showed moderate crenation. Vacuolation was present in white matter immediately adjacent to hematoma.**

for the increased sensitivity of the gradient images is the subject of subsequent investigations [5].

Neither the gradient-echo nor the T2-weighted scan sequences was sensitive to the presence or absence of blood in the subarachnoid space (Figs. 3 and 4). The failure of the T2-weighted SE scans may be due to flow artifacts in the CSF blood mixture and/or to the increase in the spectroscopically measured T2 values of blood, which occur when it is diluted with CSF [11]. The susceptibility artifacts induced by the gradient-echo scans may explain the failure of this sequence to show subarachnoid blood in cat 5 (Fig. 4). It is suggested that the detection of hyperacute subarachnoid blood was not possible at 1.5 T with the pulse sequences used in this study.

In conclusion, this experimental model demonstrated that clotted, fully deoxygenated blood may appear isointense relative to the surrounding brain on long TR, long TE MR scans done at 1.5 T within the first 10 hr after blood enters brain. The factors that eventually reduce the signal of blood below the level of the surrounding brain by causing additional shortening of T2 relaxation time of blood require further investigation. The death of one of our cats during this experiment suggests that local brain anoxia and/or hypoxia may be responsible for some of these additional changes. The inability of the MR scans to detect subarachnoid blood appears to be adequately explained by well-defined mechanisms.

ACKNOWLEDGMENTS

We appreciate the technical help of Linda Bogue, Cynthia E. Brewer, Les Smith, and Elizabeth Berry; and we are grateful for the secretarial assistance of Jackie Bogan. We thank John J. Pagani for critical comments during experimental design and manuscript preparation.

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