Journal of Magnetic Resonance **140,** 264–273 (1999)
Article ID jmre.1999.1836, available online at http://www.idealibrary.com on IDEAL®

T_1 of ^{129}Xe in Blood and the Role of Oxygenation

Mitchell S. Albert,*,[1] Daniel F. Kacher,*,† Dilip Balamore,‡ Arvind K. Venkatesh,*,† and Ferenc A. Jolesz*

*Department of Radiology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; †Department of Biomedical Engineering, Boston University, Boston, Massachusetts 02115; and ‡Department of Engineering/Physics/Technology, Nassau Community College, Garden City, New York 11530

Received November 18, 1998; revised April 14, 1999

In previous experiments by the authors, in which hyperpolarized ^{129}Xe was dissolved in fresh blood samples, the T_1 was found to be strongly dependent on the oxygenation level, the values increasing with oxygenation: T_1 was about 4 s in deoxygenated samples and about 13 s in oxygenated samples. C. H. Tseng *et al.* (1997, *J. Magn. Reson.* 126, 79–86), on the other hand, recently reported extremely long T_1 values using hyperpolarized ^{129}Xe to create a "blood foam" and found that oxygenation *decreased* T_1. In their experiments, the continual and rapid exchange of hyperpolarized ^{129}Xe between the gas phase (within blood-foam bubbles) and the dissolved phase (in the skin of the bubbles) necessitated a complicated analysis to extract the effective blood T_1. In the present study, the complications of hyperpolarized ^{129}Xe exchange dynamics have been avoided by using thermally polarized ^{129}Xe dissolved in whole blood and in suspensions of lysed red blood cells (RBC). During T_1 measurements in whole blood, the samples were gently and continuously agitated, for the entire course of the experiment, to avert sedimentation. Oxygenation was found to markedly increase the T_1 of ^{129}Xe in blood, as originally measured, and it shifts the RBC resonance to a higher frequency. Carbon monoxide has a similar but somewhat stronger effect.

Key Words: **xenon; hyperpolarized ^{129}Xe; blood; hemoglobin; paramagnetic.**

INTRODUCTION

Hyperpolarized ^{129}Xe MRI, employing as it does a new source of contrast, may facilitate the study of organs that have been difficult to image in the past (*1*). Further, it may result in new techniques for measuring cerebral blood flow and for performing functional activation studies of the brain; a chemical shift image of the ^{129}Xe distribution in the rat brain has already been demonstrated by Swanson *et al.* (*2*). Inhaled hyperpolarized ^{129}Xe rapidly diffuses into the pulmonary circulation and is then distributed throughout the body. The effectiveness of the hyperpolarized ^{129}Xe technique in imaging distal organs will depend on whether the lifetime of the hyperpolarization, *i.e.*, the longitudinal relaxation time (T_1) of ^{129}Xe in blood, is sufficiently long.

In previous experiments, in which a bolus of hyperpolarized ^{129}Xe was bubbled into fresh blood samples, we found a strong dependence of T_1 on the oxygenation level of the blood, with the values increasing with oxygenation: T_1 was about 4 s in deoxygenated samples and about 13 s in oxygenated samples (*3*). Our experimental design minimized exchange of xenon between the gas phase and the dissolved phase during the measurement stage which followed the end of the the gas bolus stage. Tseng *et al.* (*4*), on the other hand, recently reported extremely long T_1 values for xenon dissolved in blood. Their values were deduced from experiments where hyperpolarized ^{129}Xe was used to create a "blood foam." In some cases they found that T_1 was actually longer in blood than in plasma, and in general they found that oxygenation *decreased* T_1. In their experiments, the continual and rapid exchange of hyperpolarized ^{129}Xe between the gas phase (within blood-foam bubbles) and the dissolved phase (in the skin of the bubbles) necessitated a complicated analysis to extract the effective blood T_1 values. A clearer understanding of the details of the exchange of hyperpolarized ^{129}Xe between the blood and gas phase in foams is needed before a meaningful interpretation of their results can be made.

In the experiments we describe below, we have avoided the complications of hyperpolarized ^{129}Xe exchange dynamics by using ^{129}Xe at thermal equilibrium polarization, dissolved in whole blood, and in suspensions of lysed red blood cells (RBC). The samples were well mixed and the experiments were performed at equilibrium xenon concentrations. During T_1 measurements in whole blood, the samples were gently and continuously agitated, over the entire course of the experiment, to avert sedimentation. In lysed RBC suspensions there is little sedimentation during the long acquisition time required when using thermally polarized ^{129}Xe.

In the present study, we find that oxygenation increases the T_1 of ^{129}Xe in blood, confirming the trend found in Ref. (*3*). Carbon monoxide has a similar but somewhat stronger effect. Possible mechanisms for these effects are examined and the implications of these results for hyperpolarized ^{129}Xe MRI studies are discussed.

[1] To whom correspondence should be addressed. E-mail: malbert@bwh.harvard.edu.

1090-7807/99 $30.00

METHODS

Blood Samples

Fresh blood was drawn from an antecubital vein of healthy volunteers into 60-mL syringes containing a few milliliters of heparin and then placed on ice. Standard hematology gas analysis was performed at 8°C on all samples immediately before and after each T_1 experiment. A pH/blood gas analyzer (Ciba–Corning Model 238, Medfield, MA) was used to measure pH, pCO_2, pO_2, HCO_3, and sO_2. Hematocrit values were 0.045 ± 0.03. Lysed RBC suspensions and whole blood samples were prepared in both their oxygenated and deoxygenated states. Samples of lysed blood were produced by centrifugation of whole blood at 3000*g* for 10 min at 4°C to sediment the RBCs. The RBC pellets were then resuspended in 20 mM Tris buffer solution, at pH 7.6, to cause hypo-osmotic lysing of the cells. Forty-five milliliters of each sample was placed inside 85-mL glass ampoules (4 cm diameter, 7 cm length) fitted with high-pressure O-ring valves. Oxygenated samples of both lysed RBCs and whole blood were produced by gently bubbling the samples with air for about 10 min, the aeration being done in an open system at ambient pressure. The pO_2 for the oxygenated samples was measured to be 100 mm Hg at the beginning and end of the experiments. The pO_2 in alveoli is 100 mg Hg, while in arterial blood it is 95 mm Hg (*5*). Deoxygenated samples were treated by gentle and controlled bubbling of nitrogen through them until a pO_2 of 30 mm Hg was achieved. The level of oxygenation was further reduced by placing the samples under reduced pressure. The pO_2 measured at the beginning and at the end of the T_1 experiments was 20 mm Hg. Normal venous blood has a pO_2 of 40 mm Hg, and can be as low as 20 mm Hg in capillary blood, in active muscle (*5*). Whole blood samples treated with carbon monoxide were prepared by gentle bubbling of CO through the blood for 10 min. The pH values of the samples, measured before and after the experiments, were not significantly different: oxygenated samples were 7.35 ± 0.04 before and 7.37 ± 0.04 after; deoxygenated samples were 7.35 ± 0.02 before and 7.32 ± 0.13 after; CO-treated samples were 7.3 ± 0.2 before and 7.28 ± 0.2 after. To ensure that the procedures employed during sample preparation (*e.g.*, gently bubbling with nitrogen) caused no significant production of methemoglobin, in separate experiments, oxygenated and deoxygenated blood samples receiving treatment identical to those described above were measured to contain $<1\%$ (untraceable) methemoglobin, using a high-resolution spectrophotometer (Hitachi V2000, San Jose, CA).

The remaining space in the sample ampoules was then filled with xenon. A glass protrusion, or "cold finger," extending from the ampoule, was cooled by slowly submersing it in liquid nitrogen. Xenon, of natural isotopic abundance (26% ^{129}Xe), was condensed into the cold finger, the ampoules were sealed, and the xenon was allowed to evaporate and dissolve into the blood and RBC samples. A final pressure of about 3–4 atm of xenon was attained in each ampoule. In order to test whether the high pressure of xenon significantly altered T_1, additional samples were prepared at a pressure of only 1.5 atm, using isotopically enhanced xenon (70% ^{129}Xe) to counterbalance the lower signal-to-noise ratio.

In a separate experiment, carbon monoxide was gently bubbled through freshly drawn whole blood samples for 10 min, the ampoules being then pressurized with xenon at natural abundance, as described above. The pO_2 was measured in similarly prepared samples, the measurements being made at different times during the treatment with CO. On treatment with CO, the pO_2 values rose from an initial value of about of 30 mm Hg until a maximum of 300 mm Hg was reached, thereupon the values declined to about 75 mm Hg.

T_1 Measurement

^{129}Xe spectra were obtained at 55.35 MHz on a 4.7-T Bruker (Freemont, CA) Omega instrument using a 5-cm-diameter solenoid coil; the 90° pulse width was 50 μs. A spectral window of 10 kHz was used with the receiver frequency centered between the plasma and RBC resonances in whole blood, and on the single resonance from the lysed RBC samples. A 5-kHz bandpass butterworth filter was centered on the receive frequency. The samples were oriented with the length of the cylindrical ampoule perpendicular to the B_0 field.

To inhibit the auto-oxidation of hemoglobin to the paramagnetic methemoglobin form, the temperature of the sample was maintained at 8°C by flowing through the magnet bore a cold stream of nitrogen produced by evaporation of liquid N_2. The temperature was continuously monitored using an RTD sensor attached to a temperature controller (Omega 8501, Omega Instruments, Stamford, CT). In whole blood samples, the settling of RBCs was forestalled by gentle agitation. An eccentric pin on a wheel, driven by a DC gearhead motor mounted well outside the bore of the magnet, was attached to a 175-cm dowel, the other end of which was connected to the sample/coil assembly, suspended by elastic bands inside the bore. To ensure that the 2-Hz oscillations, with an amplitude of 1 cm, did not cause significant lysing of the RBCs, the whole blood samples were inspected by microscopy and by hemoglobin spectrophotometry, before and after the experiments. RBC counting of the blood was performed on a hemocytometer using a light microscope, and spectrophotometry of the plasma component was performed using the high-resolution spectrophotometer.

It is important to keep the total experiment time to well within the limits imposed by the instability of the blood samples. Exceedingly long experiment times are commonplace in measurements of T_1 of ^{129}Xe in blood, with conventional techniques using thermally polarized ^{129}Xe; this is owing to the modest value of the blood/gas partition coefficient (0.17 (*6*)), the low sensitivity of the ^{129}Xe nucleus (2.1×10^{-2} relative to ^{1}H (*7*)), and the typically long T_1 value of ^{129}Xe. We chose to use the Look–Locker single-scan inversion-recovery sequence

(*8, 9*), since it is about two to three times faster than the conventional inversion-recovery (IR) method. Look–Locker sampling is also more efficient than saturation-recovery and progressive-saturation methods (*9, 10*).

The Look–Locker method is described by the pulse sequence

$$\{180°–[\tau–\theta\,(\mathrm{acq})]_N\}_X, \qquad [1]$$

where, after the inverting 180° pulse, the recovery of the magnetization is sampled by a series of N equally spaced RF pulses of flip angle θ ($\theta < 90°$; typically ~30°) and pulse spacing τ seconds (*9*). The entire sequence is repeated x times for signal averaging. The optimal value of θ is the result of a compromise: small values yield low signal intensity, but large values too greatly reduce the z magnetization. Kaptein *et al.* (*9*) have shown that for moderate flip angles, an analysis of the relaxation of the magnetization to steady state, using the Bloch equations, gives

$$\frac{1}{T_1'} = \frac{1}{T_1} - \frac{\ln\cos\theta}{\tau} = \frac{1}{T_1} + \frac{1}{T_c}, \qquad [2]$$

where T_1' and T_1 are the apparent and true relaxation times, and T_c is the relaxation time that would result from the sampling alone, in the absence of any other relaxation. Obviously, an accurate estimate of the flip angle is critical. The solenoid coil we used was carefully tested for high homogeneity, by another research team, for use in their experiments where hyperpolarized ^{129}Xe was employed to measure diffusion of the gas (*11*). In such experiments there are stringent constraints on the tolerable level of inhomogeneity. One-dimensional projections of a ^{129}Xe gas phantom along each of the x, y, and z axes yielded perfectly symmetrical profiles. The flip angle was calibrated by plotting the signal versus the pulse width at constant amplitude. In addition, the flip angle was very accurately determined using a technique becoming increasingly common with hyperpolarized species. A series of pulses is applied with an interpulse delay $\ll T_1$ (*12*). The decrease in the signal owing to the destruction of magnetization by the read pulses should go as $(\cos\theta)^n$, where n equals the number of pulses. By fitting observations to this function, θ can be very accurately estimated.

Observations were made using eight 30° pulses, with equal interpulse delays. No recycle delay was employed, and hence, no T_1 recovery time was allowed before beginning the next average. Six data sets of 256 averages each were collected in 1.5 h per data set for each sample. Recovery curves were fitted with the Origin (Microcal Inc., Northampton, MA) package. A three-parameter mono-exponential was fitted using the Marquardt–Levenberg method. Corrected T_1 values were obtained using Eq. [2] above. T_1 values were calculated for each data set. To enhance the signal-to-noise ratio of the spectra and increase the certainty of the T_1 measurements, the data sets were averaged and T_1 values were also calculated from the averaged data sets. T_1 measurement began approximately 1 h after blood withdrawal.

RESULTS

The ^{129}Xe spectrum obtained from a sample of deoxygenated whole blood shows two dissolved-phase peaks at 196 and 216 ppm in Fig. 1a. The ^{129}Xe gas-phase resonance, which is beyond the spectral window, was referenced to 0 ppm in a previous acquisition. The peak at 196 ppm corresponds to ^{129}Xe dissolved in the plasma component of the blood, while the peak at 216 ppm is from ^{129}Xe in the deoxygenated RBCs. These assignments were made using xenon samples containing only plasma or RBCs. The spectrum from a suspension of lysed deoxygenated RBCs, Fig. 1b, shows only one peak at 203 ppm because the ^{129}Xe is in fast exchange between hemoglobin and saline, which are not separated by a cell membrane as they are in whole blood. Figures 1c and 1d show spectra obtained from isolated RBC and plasma components, respectively; they were used to identify the peaks from the whole blood sample. Oxygenation of whole blood samples shifted the RBC resonance by about 5 ppm to higher frequency.

The longitudinal relaxation time constant, T_1, of the lysed RBC samples increased upon oxygenation: the T_1 was 2.86 ± 0.11 s for the deoxygenated samples and 10.22 ± 0.38 s for the oxygenated samples. This increase of T_1 with oxygenation in the lysed RBC samples can be clearly seen in the stacked spectral plots of Fig. 2 and in the plots of the spectral peak area versus the pulse spacing, τ, displayed in Fig. 2c.

The T_1 values for the deoxygenated whole blood samples were 2.70 ± 0.22 s for the RBC component and 3.69 ± 0.44 s for the plasma component. In the oxygenated whole blood samples the T_1 increased to 7.88 ± 0.16 and 8.03 ± 1.39 s for the RBC and plasma components, respectively. The T_1 values of the RBC and plasma components were in reasonable agreement with each other, demonstrating that xenon is in fast exchange between these two sites with respect to T_1. The small difference in the T_1 values for the RBC and plasma components in the deoxygenated case is attributable to the noisy data for this particular measurement. Figure 3 displays stacked plots of the (a) deoxygenated and (b) oxygenated blood spectra, and Fig. 3c shows fits of the spectral peak areas, again demonstrating the trend for increasing T_1 with oxygenation.

Reducing the xenon pressure in the samples from about 3–4 to 1.5 atm did not have any observable effect on T_1 in these experiments. The oxygenated whole blood sample containing 1.5 atm of 70% ^{129}Xe gave T_1 values of 7.88 ± 0.1 and 7.39 ± 0.06 s for the RBC and plasma components, respectively. There was no substantial difference in T_1 between the values obtained at 1.5 atm and those obtained at 3–4 atm of xenon pressure.

Adding carbon monoxide to deoxygenated whole blood increased the T_1 values to even longer values than those observed for oxygenated blood. T_1 was 11.04 ± 2.33 and

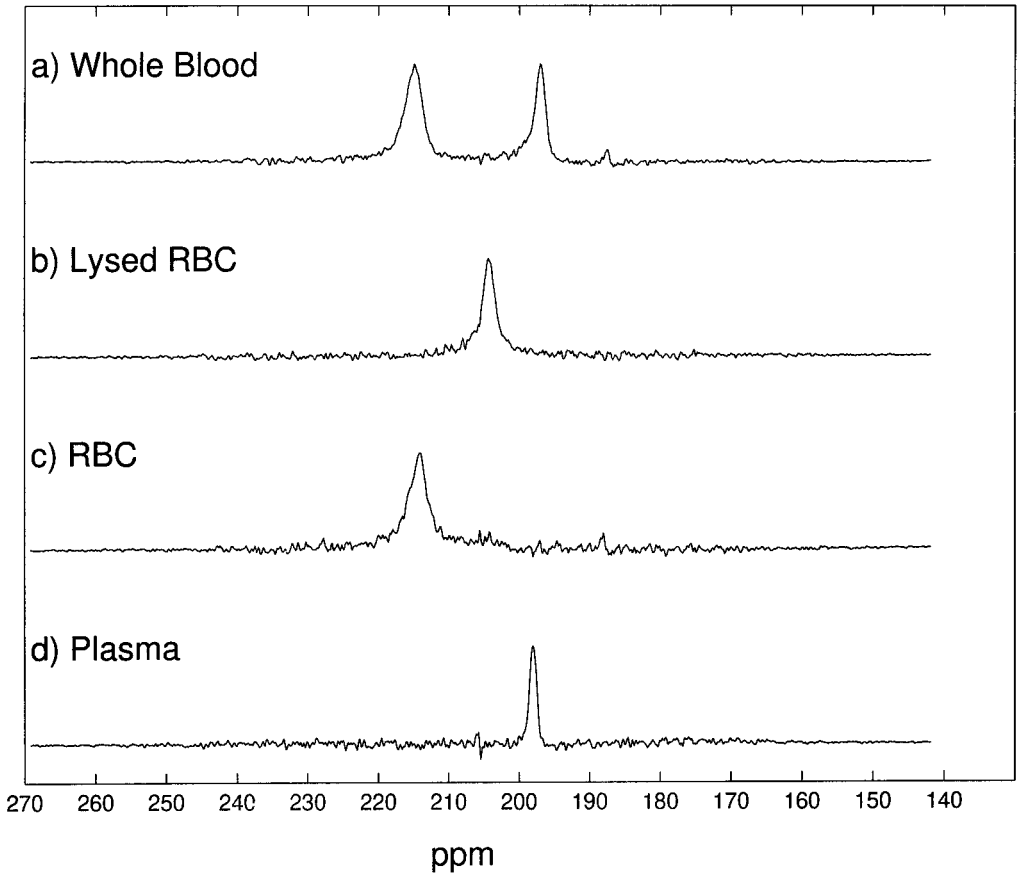


FIG. 1. Typical ^{129}Xe spectra obtained at 8°C from samples of (a) whole blood, (b) a lysed red blood cell suspension, (c) packed RBCs, and (d) plasma. Chemical shift values were referenced to the gas resonance (not shown), which was assigned to 0 ppm. Spectral signal intensities were normalized relative to the largest peak.

11.49 ± 2.92 s for the RBC and plasma components, respectively.

Spectrophotometry of the plasma components of aliquots of the whole blood samples taken before and after the experiments revealed that there was less than 5% lysing of the RBCs during the course of the experiments. Inspection of the aliquots by light microscopy confirmed this measurement. The ratio of the peak areas for the RBC and plasma signal peaks did not change significantly during the course of the experiments.

In separate experiments, whole oxygenated blood kept at 10°C for 8 h showed less than 1% conversion to methemoglobin, as measured by spectrophotometric assay. Whole oxygenated blood stored at 25°C, showed about 1% conversion to methemoglobin after 2 h, 7% conversion at 4 h, and 11.2% conversion at 6 h.

DISCUSSION

In a previous study with thermally polarized ^{129}Xe in whole blood, T_1 values of 10 and 5 s were reported for ^{129}Xe in the plasma component and in the RBC, respectively (*13*). The very different T_1 values of the two resonances are clearly due to blood sedimentation creating two pools of xenon, *not* in fast exchange. In intact whole blood one should expect a *single* T_1 value, since the ^{129}Xe will be in diffusional fast exchange, on the T_1 timescale, between the plasma and RBC components (*14, 15*). In the first part of the present study, lysed RBC suspensions eliminated RBC sedimentation, yielding a single resonance. In the whole blood experiments, sedimentation was prevented by continuous and gentle agitation. Very similar T_1 values, obtained from the RBC and plasma ^{129}Xe peaks, indicate that the exchange is, indeed, fast on the T_1 timescale. This is consistent with the results of Bifone *et al.*, who have measured the RBC/plasma exchange time to be 12 ms (*14*). In both the lysed RBC and whole blood experiments, the samples were kept at 8°C to inhibit the creation of methemoglobin (*16*), which greatly shortens T_1. *In vitro* samples are not beneficiaries of the hemoglobin reductase system of the normal erythrocyte, which *in vivo* reduces methemoglobin to hemoglobin, counteracting the continual auto-oxidation of hemoglobin to methemoglobin, and thereby limiting the methemoglobin content in circulating blood to 0.5–2% (*17*). Cooling whole blood or isolated hemoglobin, very effectively inhibits auto-oxidation: at 4°C the half-life of normal oxyhemoglobin, to auto-

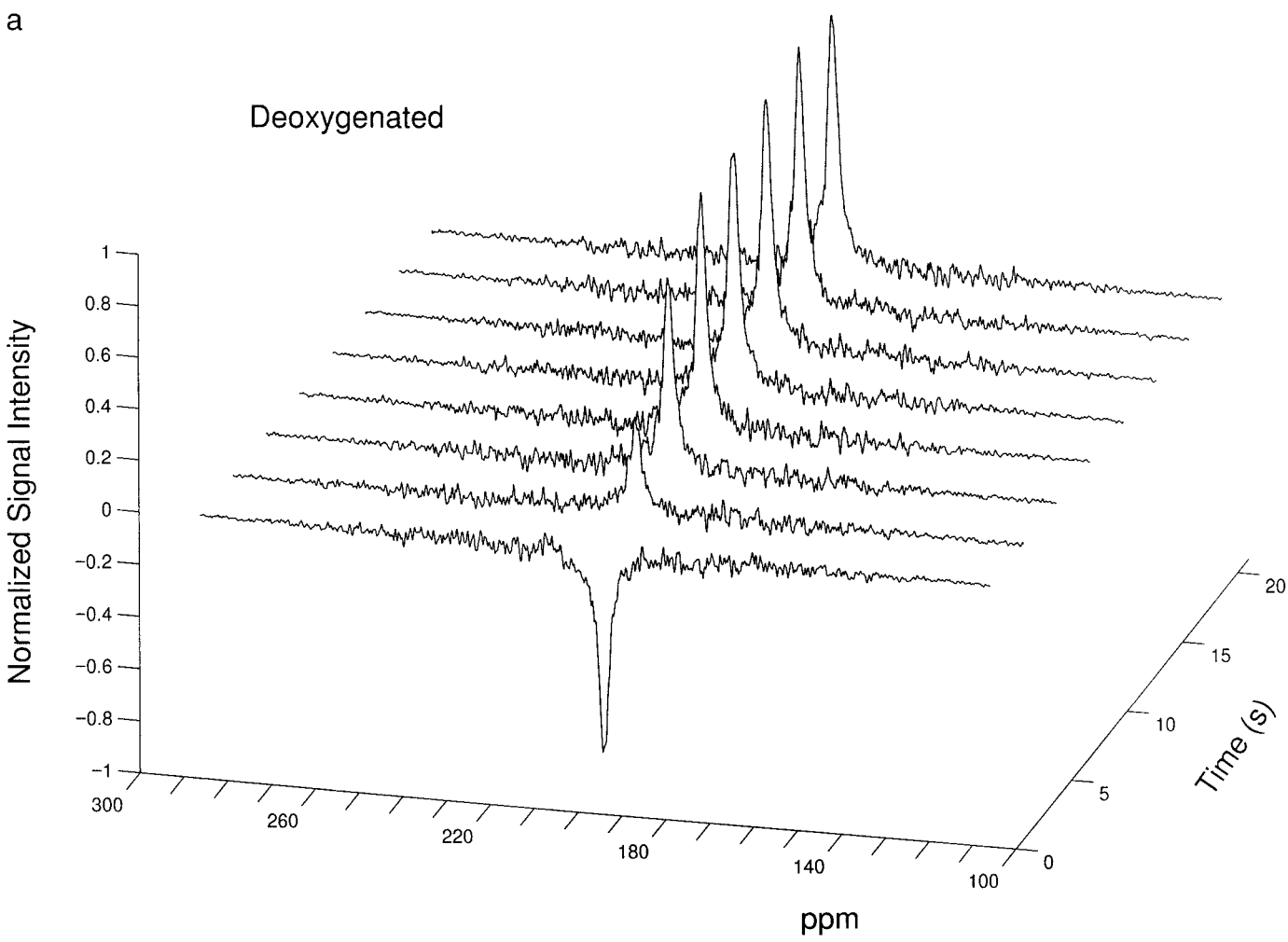
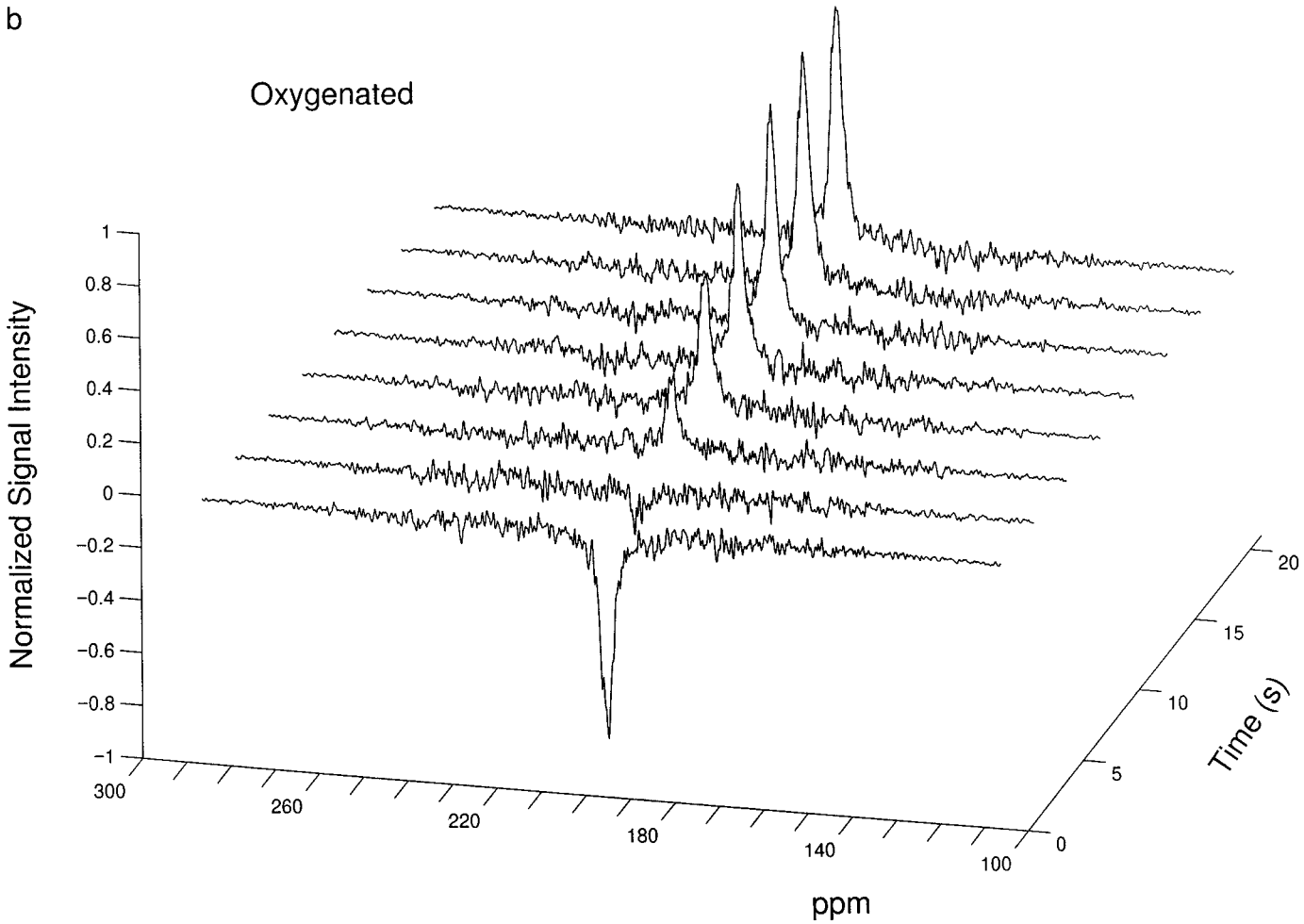


FIG. 2. Stacked plots of ^{129}Xe dissolved in samples of (a) deoxygenated and (b) oxygenated lysed red blood cell suspensions at 8°C. The data sets were acquired using a Look–Locker single-scan inversion-recovery sequence. (c) Plots of the normalized area of the peaks versus the evolution period, t, for the deoxygenated and oxygenated samples. The recovery curves show a longer T_1 value for the oxygenated suspension ($T_1 = 10.2$ s) than for the deoxygenated suspension ($T_1 = 2.95$).

oxidation, is on the order of months (*16*). We did not venture below 8°C, however, since the formation of xenon clathrates would have complicated the experiment (*18*). We note that while the actual T_1 values could possibly be different at physiological temperature (37°C) than at 8°C, the trend towards the lengthening of T_1 with oxygenation is quite robust and in conformity with our earlier room-temperature hyperpolarized ^{129}Xe observations (*3*).

The strikingly enhanced signal from hyperpolarized ^{129}Xe, dissolved in blood, reduces data acquisition times to about a minute (*3*), but the results that we obtained in the experiments described in Ref. (*3*), while they foreshadow our current findings, have nevertheless been in disagreement with the findings of Tseng *et al.* (*4*) in their research with a blood–xenon foam. Tseng *et al.* used an experimental design in which exchange effects played a most dominant role, and in which the long–lived magnetization in the gas phase continually regenerated the decaying magnetization of the xenon dissolved in blood. In their approach the extraordinarily large T_1 values they obtained were calculated by a several-parameter fit to the data. Not only are their values very different from those obtained from our two approaches, they also differ from those obtained by Bifone *et al.* (*14*). Possibly, the difficulties in modeling the exchanges between the gas and blood phases taint their results. Bifone *et al.* avoid the problem of continuing gas exchange into the blood by first dissolving hyperpolarized ^{129}Xe with saline, and then injecting it into venous blood in a tall open cylinder, the measurements being made far from the surface in order to obviate gas escape effects (*14*). Their T_1 result of 5 s in venous blood samples agrees with our findings using deoxygenated blood.

The T_1 values obtained in our present experiment must be treated with moderate guardedness: some degree of chemical change may have occurred (*16*). We note, however, that less than 1% conversion to methemoglobin was observed to occur in oxygenated blood kept at 10°C for 8 h. Blood degradation



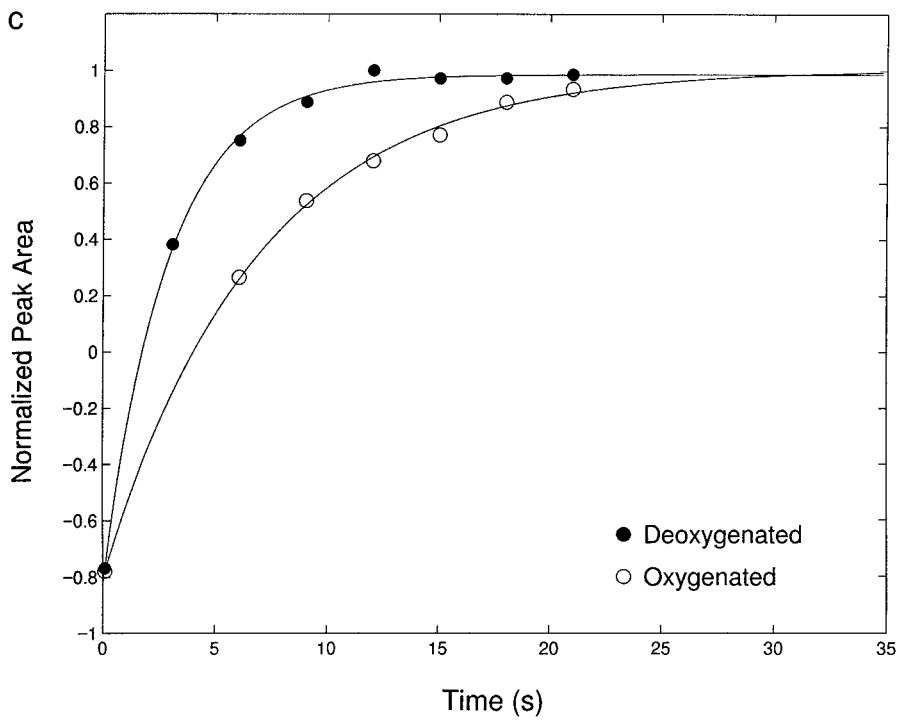


FIG. 2—*Continued*

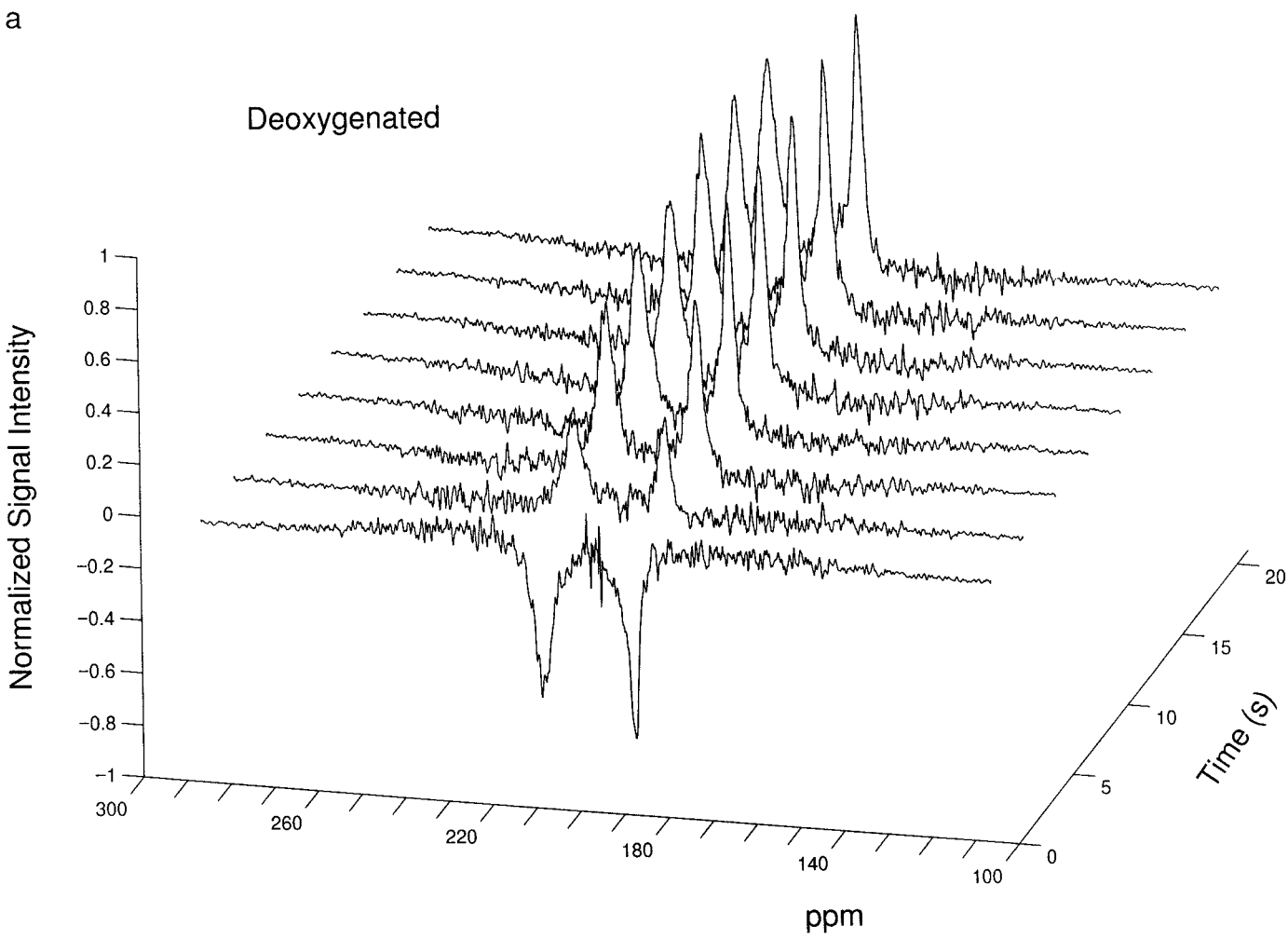
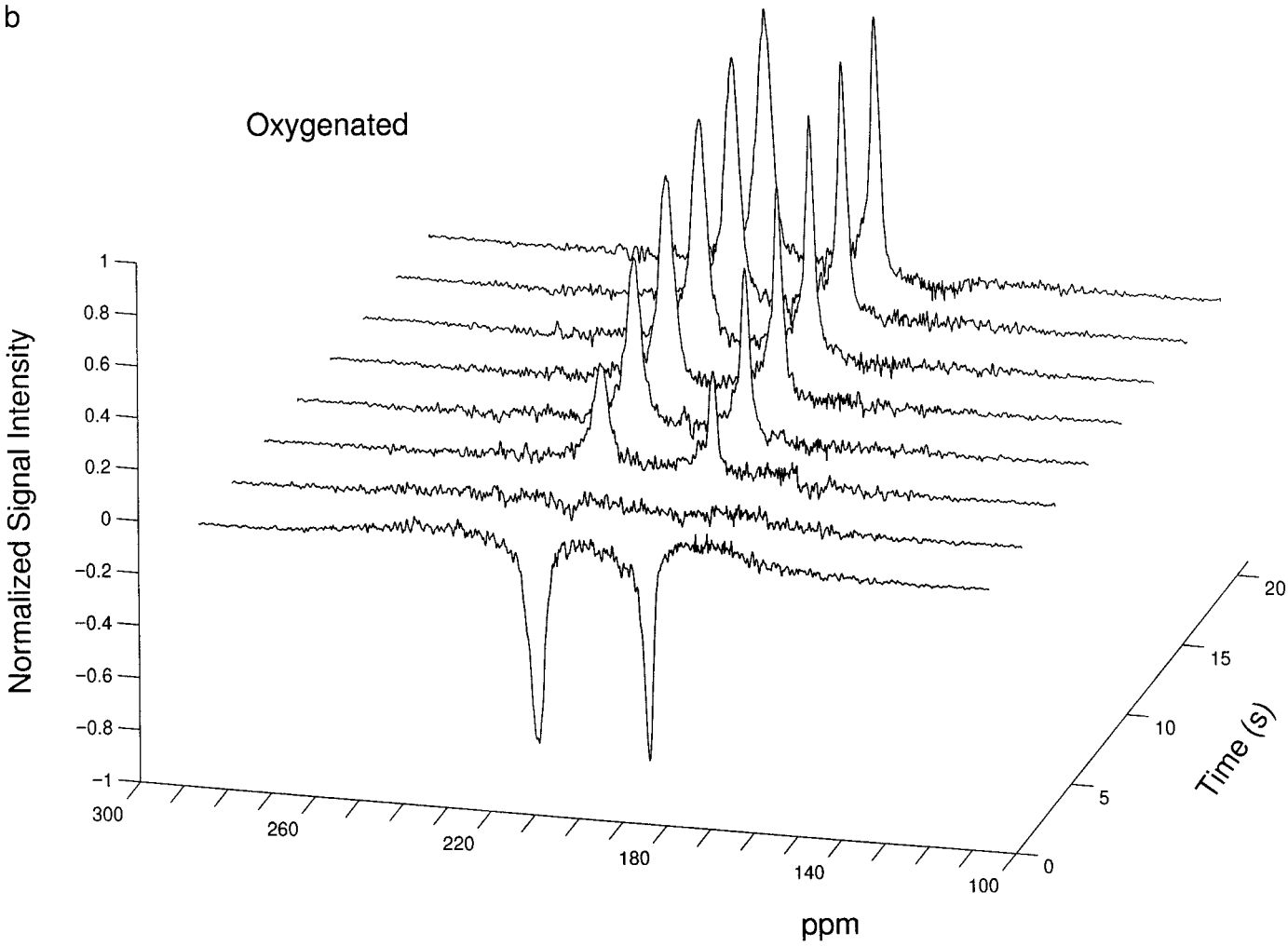


FIG. 3. Stacked plots of ^{129}Xe dissolved in (a) deoxygenated and (b) oxygenated whole blood samples cooled to 8°C. The data sets were acquired using a Look–Locker single-scan inversion-recovery sequence. T_1 values were calculated for RBC and plasma resonances. (c) Consistent with the lysed samples, the oxygenated values for both RBC (upper plot) and plasma (lower plot) were longer than those of the deoxygenated samples. T_1 values were calculated to be 2.7 s for the deoxygenated RBC component, 3.7 s for the deoxygenated plasma component, 7.9 s for the oxygenated RBC component, and 8.0 s for the oxygenated plasma component.

generally shortens T_1; consequently, it is reasonable to suggest that our measurements represent lower limits for the actual T_1 values. Our experiment was principally designed to determine the effects of oxygenation on the T_1 of ^{129}Xe in blood, and our important and robust finding has been that the longitudinal relaxation time is decidedly increased by oxygenation, being more than twice that in deoxygenated samples. The relaxation time is even longer in samples where the hemoglobin was converted into carbonmonoxyhemoglobin by treatment with CO.

Molecular oxygen is an efficient relaxation agent for ^{129}Xe in the gaseous state (*19*) and in solution (*20*). When blood is oxygenated, however, the relaxivity contribution of the unbound, paramagnetic O_2 dissolved in the blood appears to be masked by the reduction of the much greater relaxivity contribution of deoxyhemoglobin. It is interesting to note, in this context, that at physiological levels of oxygenation the ratio of O_2 chemically bound to hemoglobin to that of O_2 physically dissolved in blood is about 100:1 (*21*).

The intriguing shortening of the T_1 in deoxygenated blood may be due to the presence of paramagnetic sites in deoxyhemoglobin (*22*). Pauling and Coryell have shown the magnetic susceptibility of deoxyhemoglobin to be paramagnetic, whereas oxyhemoglobin and carbonmonoxyhemoglobin molecules have zero magnetic moment (*22*). Alternatively, the environment of the xenon binding site may be affected by the configurational differences between oxy- and deoxyhemoglobin (*23*). Xenon is known to bind to hemoglobin in specific nonpolar cavities within the protein matrix that are different from the binding sites of oxygen (*15, 24*). Hemoglobin undergoes profound changes upon oxygenation, however, both in its electronic nature (*22*) and in its structure (*23*). X-ray crystallographic studies show that oxyhemoglobin (and carbonmonoxyhemoglobin) differs markedly from deoxyhemoglobin in



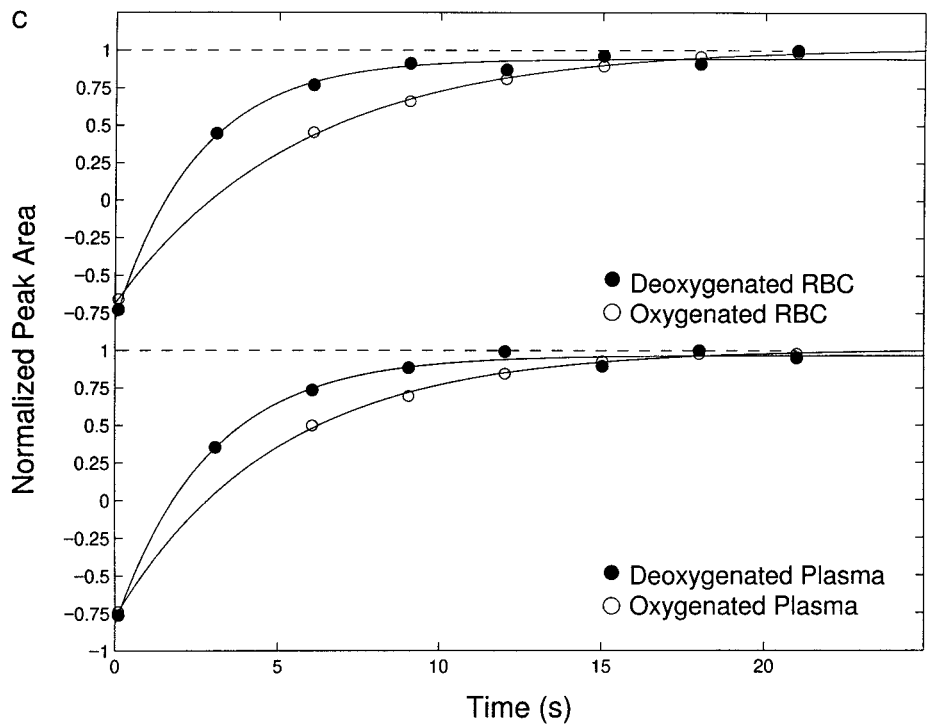


FIG. 3—*Continued*

quaternary structure (*23, 25*). Configurational changes may also affect the accessibility of the binding site and modify the residence time of xenon at the site.

It is important to note that although xenon "binds" to hemoglobin the binding is weak. Xenon binding results principally from Debye and London types of van der Waals forces, with a small contribution from an ion-induced dipole bond (*26*). Schoenborn *et al.* estimate that London interactions account for about 90% of the total binding energy (*26*). Given the extensive changes in the quaternary structure of hemoglobin upon oxygenation, the environment of the binding site may be greatly altered; indeed, it is plausible that there are different xenon binding sites in the oxygenated and deoxygenated forms. Inspection of the protein crystal structures makes it clear that the binding sites are inaccessible to ligand without cooperative protein motions to allow transient passage (*15, 24*). The structural changes produced by oxygenation may severely affect site accessibility and have a profound effect on the kinetics of binding. Altered kinetics may well explain the change in the relaxation time. CO binds more strongly than O_2, and its effects on the structure of the hemoglobin molecule are similar, but moderately stronger (*23*). This may explain why treating blood with carbon monoxide increases the T_1 of dissolved ^{129}Xe by an even greater extent than oxygenation does; T_1 values were about 8 s in oxygenated blood and about 11 s in carbonmonoxylated blood. This result *cannot* be facilely explained away as a consequence of there being little dissolved oxygen in venous blood treated with carbon monoxide. As we have described under Methods, treatment with CO displaces the considerable quantity of oxygen that is bound to hemoglobin even in venous blood, and the pO_2 value after treatment is close to that found in oxygenated blood. The longer T_1 in carbonmonoxylated blood, compared to that in oxygenated blood, may indicate differences in the *extent* of the nearly identical configurational change induced in hemoglobin by the two different ligands. Hemoglobin has an affinity for CO that is about 200 times greater than its affinity for O_2 (*5*). The oxygenation dependent shift to higher frequency of the RBC peak too may be due to these configurational differences in hemoglobin.

In our hyperpolarized ^{129}Xe studies using fresh blood at room temperature (*3*), the T_1 in oxygenated blood was found to be 13.5 s, considerably longer than the value in venous blood of 4.2 s. This *trend* has been validated by the present study using thermally polarized xenon, albeit at the reduced temperature needed to prevent degradation over the extended times required for such studies. The transport of hyperpolarized ^{129}Xe *in vivo* occurs via oxygenated arterial blood, from the lung to tissues of interest. Clearly, the relaxation time is long enough for sufficient magnetization to reach the brain (the blood travel time to the brain in the human is about 5–7s (*27, 28*)), and its strong dependence on the oxygenation state of blood bodes well for the application of hyperpolarized ^{129}Xe MRI to studies of brain function. Here, we propose two possible mechanisms by which physiological changes could produce local variations in signal intensity. First, an increase in regional cerebral blood flow should result in a corresponding increase in ^{129}Xe density in the local capillary network. Second, the corresponding increase in the oxygenation of hemoglobin might perhaps lengthen ^{129}Xe T_1 values in the local blood supply. Both of these mechanisms should function cooperatively to produce signal enhancement in local cerebral tissue. The sensitivity of the chemical shift of the RBC resonance might be exploited in order to determine the local level of oxygenation of the blood or tissue.

Finally, we note that reports of the xenon binding sites in hemoglobin have thus far been restricted to X-ray studies of Xe–hemoglobin crystal structures. The structure of Xe–hemoglobin in its biologically active liquid state may be very different from that in a crystallized form. SPINOE NMR experiments (*29*), in which some of the polarization from hyperpolarized ^{129}Xe is transferred to neighboring protons via the nuclear Overhauser effect (NOE), may show enhancement of the ^{1}H signal from protons in contact with bound hyperpolarized xenon. These types of studies should help to elucidate the changes in structure between the oxygenated and deoxygenated forms of Xe–hemoglobin in the liquid state, and aid in the determination of the mechanism responsible for the oxygenation-induced lengthening of the ^{129}Xe T_1 in blood.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (R21-RR12669), the National Science Foundation (BES-9617342), and the Whitaker Foundation (RG 95-0192). The authors thank Dr. Hao Zhu for assistance with the hemoglobin spectrophotometry measurements, Dr. Lorene Leiter for assistance with the lysed blood experiments, and Drs. H. Franklin Bunn, Kenneth R. Bridges, Chien Ho, Benno P. Schoenborn, and Max F. Perutz for helpful discussions.

REFERENCES

1. M. Albert, G. Cates, B. Driehuys, W. Happer, B. Saam, C. S. Springer, and A. Wishnia, Biological magnetic resonance imaging using laser-polarized ^{129}Xe, *Nature* **370,** 199–201 (1994).
2. S. D. Swanson, M. S. Rosen, B. W. Agranoff, K. P. Coulter, R. C. Welsh, and T. E. Chupp, Brain MRI with laser-polarized ^{129}Xe, *Magn. Reson. Med.* **38,** 695–698 (1997).
3. M. S. Albert, D. Balamore, K. Sakai, D. Kacher, R. L. Walsworth, E. Oteiza, and F. A. Jolesz, "Proceedings of the International Society of Magnetic Resonance, Fourth Scientific Meeting," p. 1357 (1996).
4. C. H. Tseng, S. Peled, L. Nascimben, E. Oteiza, R. L. Walsworth, and F. A. Jolesz, NMR of laser-polarized ^{129}Xe in blood foam, *J. Magn. Reson.* **126,** 79–86 (1997).
5. J. B. West, "Respiratory Physiology," Williams & Wilkins, Baltimore (1995).
6. R. Y. Z. Chen, F. C. Fan, S. Kim, K. M. Jan, S. Usami, and S. Chien, Tissue–blood partition coefficient for xenon: Temperature and hematocrit dependence, *J. Appl. Physiol.* **49,** 178–183 (1980).
7. R. K. Harris, "Nuclear Magnetic Resonance Spectroscopy," Longman Scienfific, Harlow, England (1986).
8. D. C. Look and D. R. Locker, Time saving in measurement of

NMR and EPR relaxation times, *Rev. Sci. Instrum.* **41,** 250–251 (1970).

9. R. Kaptein, K. Dijkstra, and C. E. Tarr, A single-scan Fourier transform method for measuring spin–lattice relaxation times, *J. Magn. Reson.* **24,** 295–300 (1976).
10. D. J. Craik and G. C. Levy, Factors affecting accuracy in ^{13}C spin–lattice relaxation measurements, *in* "Topics in Carbon-13 NMR Spectroscopy" (G. C. Levy, Ed.), Wiley, New York (1984).
11. R. W. Mair, D. G. Cory, S. Peled, C. H. Tseng, S. Patz, and R. L. Walsworth, Pulsed-field-gradient measurements of time-dependent gas diffusion, *J. Magn. Reson.* **135,** 478–486 (1998).
12. B. R. Patyal, J. H. Gao, R. F. Williams, J. Roby, B. Saam, B. A. Rockwell, R. J. Thomas, D. J. Stolarski, and P. T. Fox, Longitudinal relaxation and diffusion measurements using magnetic resonance signals from laser-hyperpolarized ^{129}Xe nuclei, *J. Magn. Reson.* **126,** 58–65 (1997).
13. M. S. Albert, V. D. Schepkin, and T. F. Budinger, Measurement of ^{129}Xe T_1 in blood to explore the feasibility of hyperpolarized ^{129}Xe MRI, *J. Comput. Assist. Tomgr.* **19,** 975–978 (1995).
14. A. Bifone, Y. Q. Song, R. Seydoux, R. E. Taylor, B. M. Goodson, T. Pietrass, T. Budinger, G. Navon, and A. Pines, NMR of laser-polarized xenon in human blood, *Proc. Natl. Acad. Sci. USA* **93,** 12932–12936 (1996).
15. R. F. Tilton, Jr. and I. D. Kuntz, Jr., Nuclear magnetic resonance studies of xenon-129 with myoglobin and hemoglobin, *Biochemistry* **21,** 685–6857 (1982).
16. E. A. Rachmilewitz, J. Peisach, and W. E. Blumberg, Studies on the stability of oxyhemoglobin A and its constituent chains and their derivatives, *J. Biol. Chem.* **246,** 3356–3366 (1971).
17. D. W. van Assendelft, "Spectrophotometry of Hemoglobin Derivatives," Charles Thomas Pub., The Netherlands (1970).
18. T. Pietrass, H. C. Gaede, A. Bifone, A. Pines, and J. A. Ripmeester, Monitoring xenon clathrate hydrate formation on ice surfaces with optically enhanced ^{129}Xe NMR, *J. Am. Chem. Soc.* **117,** 7520–7525 (1995).
19. C. J. Jameson, A. K. Jameson, and J. K. Hwang, Nuclear spin relaxation by intermolecular magnetic dipole coupling in the gas phase, *J. Chem. Phys.* **89,** 4074 (1988).
20. M. S. Albert, C. S. Springer, and A. Wishnia, Abstracts of the Society of Magnetic Resonance in Medicine, 11th Annual Meeting, p. 4710 (1992).
21. A. J. Vander, J. H. Sherman, and D. S. Luciano, "Human Physiology: The Mechanics of Body Function," McGraw–Hill, New York (1975).
22. L. Pauling and C. D. Coryell, The magnetic properties and structure of hemoglobin, oxyhemoglobin and caron monoxyhemoglobin, *Proc. Natl. Acad. Sci. USA* **22,** 210–216 (1936).
23. R. E. Dickerson, "Hemoglobin, Structure, Function, Evolution and Pathology," Benjamin Cummings Pub., Menlo Park, CA (1983).
24. B. P. Schoenborn, Binding of xenon to horse haemoglobin, *Nature* **208,** 760–762 (1965).
25. G. Fermi and M. F. Perutz, "Hemoglobin and Myoglobin," Oxford Univ. Press, New York (1981).
26. B. P. Schoenborn, A. Saxena, and B. E. North, Weak binding gases as modulators of hemoglobin function, *in* "The Molecular Basis of Mutant Hemoglobin Dysfunction" (P.B. Sigler, Ed.), Elsevier/North-Holland, Amsterdam (1981).
27. H. L. Blumgart and S. Weiss, The pulmonary circulation time in normal resting individuals, *J. Clin. Invest.* **4,** 399–425 (1927).
28. R. M. Berne and M. N. Levy, "Cardiovascular Physiology," Mosby, St. Louis (1977).
29. G. Navon, Y. Q. Song, T. Room, S. Appelt, E. Taylor, and A. Pines, Enhancement of solvent NMR and MRI by hyperpolarized ^{129}Xe, *Science* **271,** 1848 (1996).