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OXYGENATION DEPENDENCE OF THE TRANSVERSE RELAXATION TIME OF WATER PROTONS IN WHOLE BLOOD AT HIGH FIELD

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At high and medium magnetic field, the transverse NMR relaxation rate (T_2^{-1}) of water protons in blood is determined predominantly by the oxygenation state of haemoglobin. T_2^{-1} depends quadratically on the field strength and on the proportion of haemoglobin that is deoxygenated. Deoxygenation increases the volume magnetic susceptibility within the erythrocytes and thus creates local field gradients around these cells.From volume susceptibility measurements and the dependence of T_2^{-1} on the pulse rate in the Carr-Purcell-Meiboom-Gill experiment, we show that the increase in T_2^{-1} with increasing blood deoxygenation arises from diffusion of water through these field gradients.

Introduction

There is considerable interest in NMR spectroscopy of animals and humans in vivo. Anatomical information can be obtained from proton imaging (zeugmatography) [1]. This method relies on the differences in the volume fraction and spin-lattice relaxation of water amongst different tissues and between different physiological states of the same tissue. A second approach, high-resolution ^{31}P-NMR [2], seeks to determine the intracellular pH and the concentration and dynamics of certain phosphate metabolites to reveal directly the metabolic state of the tissue. We have now observed that the proton transverse relaxation time (T_2) of water in the blood provides additional important physiological information, namely the oxygenation state of the haemoglobin in whole blood. From the NMR measurement of blood oxygenation [3] combined with the NMR measurement of blood flow [4,5], oxygen consumption by blood-perfused or in vivo tissues and organs can be continuously monitored [3]. Thus, the information derived from this new experiment is complementary to the bioenergetic information obtained from high-resolution ^{31}P-NMR and the structural details obtained from ^{1}H imaging.

In this paper we examine the origin of the dependence of T_2 on the oxygenation state of blood.

Materials and Methods

Blood was freshly drawn by cardiac puncture from heparinized male Wistar rats (250–350 g) anaesthetised by intraperitoneal injection of sodium pentabarbitone (0.2 ml, 60 $mg^{-1} \cdot ml^{-1}$) and stored on ice until used. Rabbit blood was obtained in the same manner from New Zealand White rabbits (3 kg) after intravenous injection of the above anaesthetic. The haematocrit was measured before and after each

Abbreviations: T_1, longitudinal relaxation time; T_2, transverse relaxation time; H_0, static magnetic field strength; CPMG, Carr-Purcell-Meiboom-Gill pulse sequence; $2\tau_{CPMG}$, interval between 180° pulses in the CPMG experiment; χ_V, volume magnetic susceptibility; Hb, deoxygenated haemoglobin; HbO_2, oxygenated haemoglobin.

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experiment on a Hawkesley Microhaematrocrit Centrifuge and showed little variation (40 ± 5%) over seven experiments. Blood oxygenation was increased from the low level (25 ± 5%) at which it was drawn by swirling the blood gently under a stream of oxygen gas. Lower oxygenation states were achieved by the addition of a few grains of sodium dithionite (BDH Ltd.). This treatment was completely reversible if only small quantities of sodium dithionite were used. The oxygenation state was measured using a calibrated haemoreflector (Kipp and Zonen). Whole blood lysate was prepared by two freeze-thaw cycles. Lysate of packed cells was produced by centrifugation at 10000 × g for 20 min followed by lysis of the pellet by two freeze-thaw cycles. The haematocrit was varied by mixing different proportions of blood and plasma.

The NMR measurements, observing the water proton resonance, were made using the following spectrometer systems: a Varian T-60 at 60 MHz; an Oxford Research Systems TMR-32 magnet interfaced to a Nicolet 1180-293A′ computer and operating at 80.2 MHz; a Bruker WM-400 at 400.1 MHz; and three spectrometers built in this laboratory, interfaced to Nicolet 1180-293A′, Nicolet 1180-293B and Nicolet 1180-293A computers operating at 182.4, 270 and 469 MHz, respectively.

Transverse relaxation time (T_2), longtudinal relaxation times (T_1) and volume magnetic susceptibilties were measured. In the cases where a Nicolet 1180 system was used, values of T_2 were measured by a Carr-Purcell-Meiboom-Gill (CPMG) sequence [6]: $90^\circ_x - \tau_{CPMG} - 180^\circ_y - \tau_{CPMG})_n$; where τ_{CPMG} = 1 ms (except where otherwise stated), n = 512. At the end of every second τ_{CPMG} the outputs of two phase sensitive detectors, in quadrature A and B, were sampled. T_2 was calculated by a least-squares fit of $(A^2 + B^2)^{1/2}$ against time to a single exponential. Hence each measurement required less than 1 s. The remaining measurements were made by increasing $2\tau_{CPMG}$ and Fourier transformation of the terminal half echo. In experiments where $2\tau_{CPMG}$ was varied, it was not possible to set $2\tau_{CPMG}$ to less than 1 ms for instrumental reasons. Values of T_1 were measured by the inversion-recovery Fourier transform technique without correction for the effects of radiation damping. Bulk susceptibilities were measured by the method of Becconsall et al. [7]. All values are quoted as ± one standard deviation of the mean. Except where otherwise stated, the measurements were made at 37°C.

Results

Relaxation times

Fig. 1 shows T_1^{-1} and T_2^{-1} for rat blood plotted against (fraction blood deoxygenated)^2 demonstrating a quadratic dependence for T_2^{-1}. A similar dependence was observed for blood from rabbits (see Table I). Table I shows the dependence of T_2^{-1} on the spectrometer frequency, υ_0, while Fig. 2 plots log-($T_2^{-1}{}_{ex}$) against log(H_0) demonstrating a quadratic relationship between $T_2^{-1}{}_{ex}$ and the static field strength. $T_2^{-1}{}_{ex}$ is defined in this experiment as: $T_2^{-1}{}_{ex} = T_2^{-1}$ (venous) $- T_2^{-1}$ (arterial) where venous blood was 20–30% oxygenated and arterial blood was 95–100% oxygenated. Fig. 3 present the variation of $T_2^{-1}{}_{ex}$ with $2\tau_{CPMG}$ at two levels of oxygenation. The curves are theoretically derived as explained in the discussion.

For whole blood lysate at 182.4 MHz, T_2^{-1} (oxy) = 18.5 s^{-1} while T_2^{-1} (deoxy) = 14.5 s^{-1}.

Constant oxygenation state at low haematocrit was difficult to maintain. The haematocrit dependence of T_2^{-1} for oxygenated blood (95–100%) is linear, while for deoxygenated blood (10–20%) the relationship is more complex (Fig. 4). The solid lines represent theo-

TABLE I

FREQUENCY DEPENDENCE OF T_2^{-1} FOR BLOOD (NORMAL HAEMATOCRIT, 37°C)

Values of T_2^{-1} are mean ± S.D. for at least three measurements.

° (MHz)	T_2^{-1} (oxy) (s^{-1})	T_2^{-1} (deoxy) (s^{-1})
Rat		
80.3 [a]	5.5 ± 0.05	13.8 ± 0.01
182.4 [b]	6.9 ± 0.05	51 ± 5
270 [a]	10.9 ± 0.1	67 ± 8
469 [a]	15.4 ± 0.1	111 ± 25
Rabbit		
80.2 [b]	4.3 ± 0.05	12.5 ± 0.1

[a] From measurements of blood at 100% and less than 25% oxygenation.

[b] Extrapolated from full calibration curve (see Fig. 1).

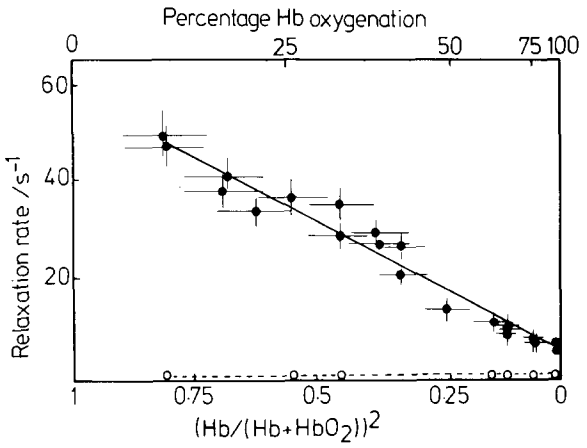

Fig. 1. Dependence of relaxation rates, T_2^{-1} (•) and $T_1{}^1$ (○), on and the square of the fraction of deoxygenated blood. The error bars represent ±10% in T_2 and ±5% in % oxygenation. The lines were fitted with a least-squares fitting routine.

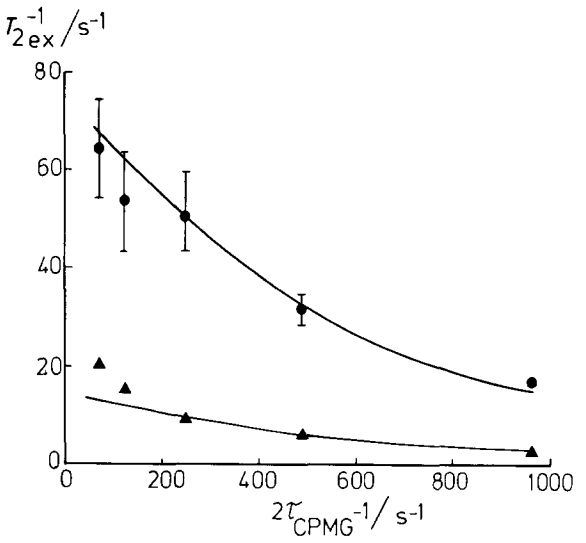

Fig. 3. Variation of $T_{2\,ex}^{-1}$ with CPMG pulse rate for oxy (•) and and deoxy (▲) blood. The theoretical curves are discussed in the text. Representative errors bars are shown and indicate that the points on the left of the figure are least reliable as they correspond to the smallest number of spin echoes and so are determined with the least precision. Deviations from theory are also likely for these values.

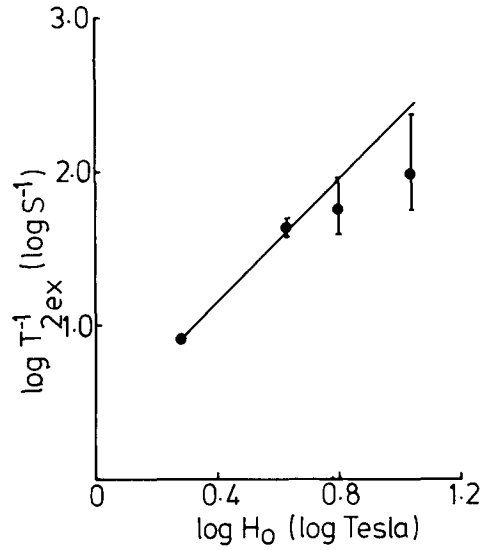

Fig. 2. Variation of $T_{2\,ex}^{-1}$ with H_0 on logarithmic scales. Data taken from Table I. The typical error bars for the measurements at each field strength are indicated. The high field values are the least accurate because of the small T_2 values relative to the $2\tau_{CPMG}$ = 2 ms. Small variations in the measurement of oxygenation also contribute significantly at high field to reduce $\log(T_{2\,ex}^{-1})$. The line has a gradient of 2 when drawn through the two most accurate values measured at 182.4 and 80.29 MHz.

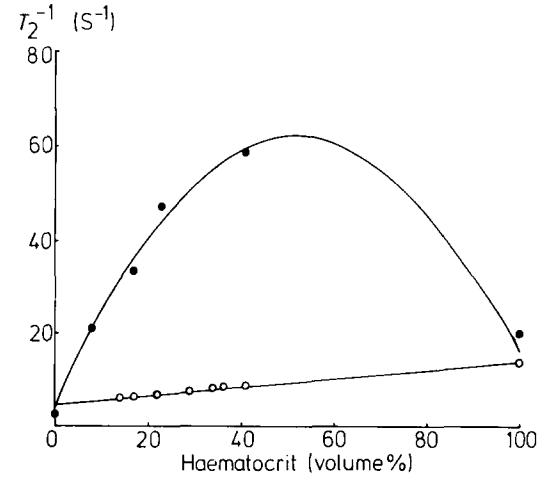

Fig. 4. The haematocrit dependence of the water proton relaxation time, T_2^{-1}, at a field strength of 4.28 Tesla for oxy (○) and deoxy (•) blood. The curves are theoretical as discussed in the text.

retical curves as described in the discussion. Over the physiological range of haematocrits (30–50%), relatively little variation (±15%) in T_2 was observed. Similarly, the temperature variation in T_2 was less than 3% over the range 20–37°C.

Volume susceptibilities

The resonance frequency of protons of whole blood decreased linearly with deoxygenation at 270 MHz (cylindrical samples parallel to H_0) with the shift being 89 Hz over the full oxygenation range. Thus, if changes in volume susceptibility χ_v, were the only cause of the shift, then, for whole blood $\Delta\chi_v = \chi_v$ (deoxy) $- \chi_v$ (oxy) $= 0.08 \cdot 10^{-6}$. Assuming that χ_v for plasma was unchanged on deoxygenation, then $\Delta\chi_v = 0.2 \cdot 10^{-6}$.

In a separate experiment, it was confirmed that the shift was entirely due to changes in volume susceptibility. A spinning coaxial sample with oxygenated blood in the capillary and deoxygenated blood in the annulus was examined at 400 MHz (sample parallel to H_0) and 60 MHz (sample perpendicular to H_0). Water resonances from the two compartments were readily resolved. When the susceptibility shift had been accounted for, the chemical shift difference between oxygenated and deoxygenated blood was less than 0.001 ppm.

Discussion

The erythrocyte contains a high concentration of haemoglobin iron (ca. 20 mM) which changes spin-state from diamagnetic low-spin ($S = 0$) FeII in the oxygenated state (HbO_2) to paramagnetic high-spin ($S = 2$) FeII in the deoxygenated state (Hb). This change in spin-state is the basis of the oxygenation dependence of the transverse relaxation time of water protons in blood. Possible mechanisms for this dependence are: (i) translational modulation of the proton-electron dipolar coupling; (ii) changes in proton-proton dipolar relaxation with viscosity; (iii) contact broadening; (iv) pseudocontact broadening and (v) diffusion or exchange of water between sites of different Larmor frequency arising from local variations in volume susceptibility.

We now consider these mechanisms in order:

(i) In principle the presence of a high concentration of paramagnetic centres within the erythrocyte could enhance both longitudinal and transverse relaxation rates of protons in blood water via translational modulation of the proton-electron dipolar coupling. However, because deoxygenation adds 51 s^{-1} to T_2^{-1} at 182.4 MHz while the contribution to T_1^{-1} is less than 0.2 s^{-1} (Fig. 1), we conclude that this mechanism does not account for the dependence of T_2 on oxygenation. Although the water in blood exists in two pools (intra- and extracellular) no evidence of non-exponential longitudinal relaxation was observed. This is consistent with the rapid exchange of water across the erythrocyte membrane (see below).

(ii) Similarly, any increase in local viscosity with deoxygenation [8] that might significantly affect the homonuclear dipolar contribution to T_2 would be expected to change the value of T_1 as well as T_2.

(iii) Exchange between two sites, one of which experiences a hyperfine (contact) interaction with a paramagnetic centre, can drastically reduce T_2 without affecting T_1 [9]. We reject this explanation for three reasons; water is not a ligand at the FeII centre in Hb; the iron electron T_1 [10] should be sufficiently short to collapse the hyperfine structure even in a static field of several Tesla; and the T_2 of water protons of blood lysate showed little change upon deoxygenation. In this last case, disruption of the plasma membrane disturbs the metabolism of the red blood cell but leaves functional haemoglobin.

(iv) The independence of T_2 on deoxygenation in the lysate of erythrocytes also discounts incomplete averaging of the dipolar (pseudocontact) coupling [11,12] as a possible explanation.

(v) The final mechanism is dependent upon both the integrity of the red blood cell and the spin-state of haemoglobin. The volume susceptibility of oxygenated erythrocytes containing low-spin diamagnetic haem is similar to that of blood plasma.

In contrast, the high concentration of high-spin paramagnetic FeII within the erythrocyte in the deoxygenated state increases the intracellular volume susceptibility while leaving the susceptibility of the plasma unchanged. Our results show that on deoxygenation, the intracellular volume susceptibility increases by $0.2 \cdot 10^{-6}$ while the chemical shift change is negligible. If rotation of an erythrocyte is slow on the NMR time scale, the average Larmor frequency for water protons in an erythrocyte becomes dependent on the orientation of the cells in the static

field H_0 and static field gradients are induced both inside and outside the erythrocyte [13].

In order for these field gradients to explain the decrease in T_2 on deoxygenation, there must be a mechanism for the exchange of water protons between sites of differing Larmor frequency. Three processes to consider are: (a) modulation of local field gradients by tumbling of erythrocytes; (b) exchange of water protons across the erythrocyte membrane; and (c) diffusion of water through intracellular and/or extracellular field gradients [13]. Each of these processes can, in principle, be characterized by a correlation time ξ and a distribution of Larmor frequencies ω. For each mechanism of exchange, $T_2^{-1}{}_{ex}$ should be proportional to $\xi(\Delta\omega)^2$ at a given haematocrit, where $\Delta\omega$ is the range of frequencies in the sample. This assumes that either $\xi < 2\tau_{CPMG}$ (the fast exchange condition) or the probability of a spin moving in a time $2\tau_{CPMG}$ to a site of new frequency is independent of the original frequency (the jump-diffusion condition) [14]. We have varied $\Delta\omega$ by independently altering H_0 and the degree of oxygenation. In both cases the expected quadratic dependence can be seen (Table I and Fig. 1).

For samples of different haematocrit where the distribution, rather than the magnitude, of the local fields and field gradients, is changed, we expect $T_2^{-1}{}_{ex}$ to be proportional to $\xi\Sigma_i p_i(\omega_i - \omega_0)^2$ where p_i is the population of sites of frequency ω_i, and ω_0 is the mean frequency: $\Sigma_i p_i(\omega_i - \omega_0) = 0$. Although the dependence of $T_2^{-1}{}_{ex}$ upon haematocrit was experimentally difficult to determine (as discussed under Materials and Methods) our results showed that the experimental dependence of T_2^{-1} (deoxy) on haematocrit was well described by the above theoretical treatment (see Fig. 4). T_2^{-1} (oxy) showed little dependence on the haematocrit.

The relative contributions of the three exchange processes outlined above may be assessed by considering their correlation times: (a) As the correlation time for erythrocyte rotation, estimated from the Debye Stokes equation, exceeds 10 s its contribution to $T_2^{-1}{}_{ex}$ must be negligible. (b) The correlation time for exchange across the erythrocyte membrane is known to be of the order of 10 ms [15] or longer [16]. (c) The correlation time for diffusion through local field gradients is not well-defined. However, Packer [17] has pointed out that, providing the local field gradients are linear and their periodicity is well-defined (neither condition being satisfied by whole blood) diffusion through internal field gradients is formally similar to restricted diffusion through an externally imposed static field gradient [18].

The correlation time for the former process is then the time for a water proton to experience the entire range of frequencies, $\Delta\omega$. If the periodicity of the field gradients in whole blood, r_p, is of the order of half the thickness of the erythrocyte ($0.5 \cdot 2\ \mu m$) and the self diffusion constant, D, for water has a similar value, $10^{-9}\ m^2 \cdot s^{-1}$, in blood to that measured in other tissues [17,19] then ξ for periodic diffusion [17], $r_p^2/2D$, is of the order of 0.5 ms.

We have obtained experimental estimates of ξ and $\Delta\omega$ in blood. The volume susceptibility difference $\Delta\chi_v$ between plasma and deoxygenated cytoplasm was measured to be about $0.2 \cdot 10^{-6}$, in fair agreement with a calculated value of $0.24 \cdot 10^{-6}$ based on the known susceptibility of haemoglobin [20]. $\Delta\omega/\omega$ will exceed $\Delta\chi_v$ by some geometrical factor, k:

$$(\Delta\omega)/\omega_0 = k\Delta\chi_v$$

where k depends on the shape and packing of the erythrocytes. From previous work using glass beads [21] and from well known formulae concerning coaxial cylinders [7,22] we estimate that k is, in the order of magnitude, 7. Thus, $\Delta\omega/\omega_0$ is about 1.4 ppm. We cannot measure this directly from the frequency domain NMR spectrum since the lineshape is partially collapsed by exchange. However $(\Delta\omega)/\omega_0$ must clearly exceed full linewidth at half-height, which was measured for deoxygenated blood at 182.4 MHz and 400.1 MHz to be 0.3 ppm.

We have obtained an estimate of ξ by varying $2\tau_{CPMG}$. Fig. 3 shows the dependence of $T_2^{-1}{}_{ex}$ upon $(2\tau_{CPMG})^{-1}$ for blood of the different oxygenation states. The theoretical curves were fitted using the theory of Luz and Meiboom [14] assuming that for fully deoxygenated blood $(\Delta\omega)/\omega_0 = 1.35$ ppm, the distribution of frequencies over $\Delta\omega$ was uniform, and the jump-diffusion model held. The data were best fitted for a value of $\xi = 0.6$ ms. The curvature of the theoretical plots was quite sensitive to ξ.

This value of ξ is close to that estimated for diffusion through local field gradients (0.5 ms) but rather short compared with ξ for exchange across the

membrane (10 ms). Thus, we conclude that the dependence of blood T_2 on oxygenation results from the diffusion of water protons through local field gradients arising from the increased volume susceptibility of deoxygenated erythrocyte cytoplasm.

This effect is potentially useful for determining the state of oxygenation of blood in vivo rapidly, non-invasively and quantitatively. The effect is relatively insensitive to temperature and haematocrit over the physiological range. Because of the quadratic dependence on H_0, the measurement may be made at high and medium fields, certainly at the field strength (1.9 Tesla) currently being used for clinical ^{31}P-NMR metabolic studies [23]. This measurement will not be possible at the low field strengths (less than 0.36 Tesla) being used for clinical ^{1}H-NMR imaging [1]. The feasibility of using NMR to measure both blood oxygenation and blood flow to obtain oxygen consumption has already been demonstrated in laboratory animals [3,24].

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