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Magnetization Transfer Contrast (MTC) and Tissue Water Proton Relaxation *in Vivo*

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In this study the exchange between ^1H magnetization in "free" water (^1H_f) and that in a pool with restricted motion (^1H_r) was observed in tissues *in vivo* using NMR saturation transfer methods. Exchange between these two pools was demonstrated by a decrease in the steady-state magnetization and relaxation times of ^1H_f with radiofrequency irradiation of ^1H_r. The pseudo-first-order rate constant for the movement of magnetization from ^1H_f to ^1H_r was $\sim 1\ s^{-1}$ in kidney and $\sim 3\ s^{-1}$ in skeletal muscle *in vivo*. Proton NMR imaging demonstrated that this exchange was tissue specific and generated a novel form of NMR image contrast. The extent of exchange between ^1H_f and ^1H_r as well as the topological correlation of the exchange with relaxation weighted images suggests that this pathway is a major determinant of the observed relaxation properties of water ^1H *in vivo*.

INTRODUCTION

The relaxation properties of water ^1H nuclei are the basis for most of the contrast obtained by nuclear magnetic resonance (NMR) imaging techniques. Conventional ^1H NMR images of biological tissues usually reflect a combination of spin–lattice (T_1) and spin–spin (T_2) water ^1H relaxation. The variations in water ^1H relaxation rate generate image contrast between different tissues and pathologies depending on how the NMR image is collected. While the general mechanisms for water ^1H relaxation have been described (*1*), the precise nature of these relaxation processes in tissue is still an active area of research. Investigators have proposed that a predominant relaxation mechanism in biological tissue is cross-relaxation and/or chemical exchange between ^1H in "free" or highly mobile water and ^1H associated with macromolecules or immobile water (*2–6*). These conclusions were based on detailed studies of the effect of static magnetic field strength (*5, 6*) as well as selective and nonselective excitation pulses (*2–4*) on water ^1H relaxation rates.

The purpose of this study was to measure the rate of magnetization exchange between ^1H spins in "free" water (^1H_f) and ^1H spins in regions of restricted motion (^1H_r) as well as to image the distribution of this exchange process in intact tissues. To accomplish this, we used the saturation transfer method originally described by Forsen and Hoffman (*7*) in which the rate of magnetization exchange between two

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pools can be quantitatively measured. This technique has been used extensively to measure chemical exchange rates in intact tissues (*8–10*). Saturation transfer involves the selective steady-state irradiation, with radiofrequency energy, of one member of an exchanging pair of spins and observation of the effects of this irradiation on its nonirradiated exchange partner. If exchange is occurring, the irradiation will cause a decrease in the steady-state magnetization and a decrease in the observed T_1 of the nonirradiated spin (*8, 11*). These effects are not limited to chemical exchange, since through-space cross-relaxation between the two pools will have identical effects.

MATERIALS AND METHODS

All experiments were performed using General Electric CSI spectrometers operating at 4.7 or 2.0 T. The rabbit kidney and skeletal muscle preparations were used as previously described (*9, 10, 12*). Coil designs for these studies were also previously described for the rabbit leg (*9*) and kidney (*12*) *in vivo*.

RESULTS

Saturation transfer experiments were performed in the present study by irradiating the immobilized ^1H_r pool and observing the effect on the steady-state magnetization as well as the observed spin–lattice relaxation (T_1) time of ^1H_f. We assumed that radiofrequency irradiation $\geq$5 kHz from the ^1H_f resonance would result in a selective irradiation of ^1H_r, since ^1H_r would have an NMR linewidth much greater than ^1H_f (i.e., the T_2 relaxation time should be shorter for ^1H_r) (*13*). Saturation transfer experiments were conducted by preirradiating at frequencies selected within 200 kHz of the Larmor frequency of the ^1H_f resonance. Figure 1 shows the effect of irradiation at the frequency 10 kHz lower than the ^1H_f resonance of a rabbit kidney *in vivo*. The preirradiation resulted in a specific 30% decrease in the magnitude of the water resonance (measured as the area of the resonance peak) that was not observed for the other ^1H signals from fats or trimethylamines even though the irradiation was closer in frequency to these metabolites. This effect was also reproduced in rabbit skeletal muscle and brain *in vivo*. These results are consistent with the presence of a specific magnetization exchange between ^1H_f and ^1H_r pools in these tissues.

The decrease in ^1H_f signal by irradiation of ^1H_r was found to be power- and frequency-dependent. The effect of irradiation power on the steady-state ^1H_f signal is shown in Fig. 2A. Using this curve, the maximum decrease in steady-state ^1H_f magnetization obtained by irradiating ^1H_r was estimated to be 70% in the kidney *in vivo*. This irradiation power versus ^1H_f magnitude curve was obtained for all of the tissues and fluids studied in this investigation to assure that a maximum effect of irradiation was recorded at any given frequency.

A significant decrease in the net magnetization of the ^1H_f resonance was observed over a wide range of irradiation frequencies ($\pm$50 kHz) surrounding its resonance frequency. The effect of different irradiation frequencies, at constant power (0.4 W), is shown in Fig. 2B. The linewidth of the irradiation effect was approximately 40 kHz and symmetrically centered around the ^1H_f resonance in the rabbit kidney (Fig. 2B) and skeletal muscle *in vivo* (not shown) as well as in canine heart muscle *in vitro* (Fig. 2C). No magnetization transfer was observed in distilled water (Fig. 2B), manganese-

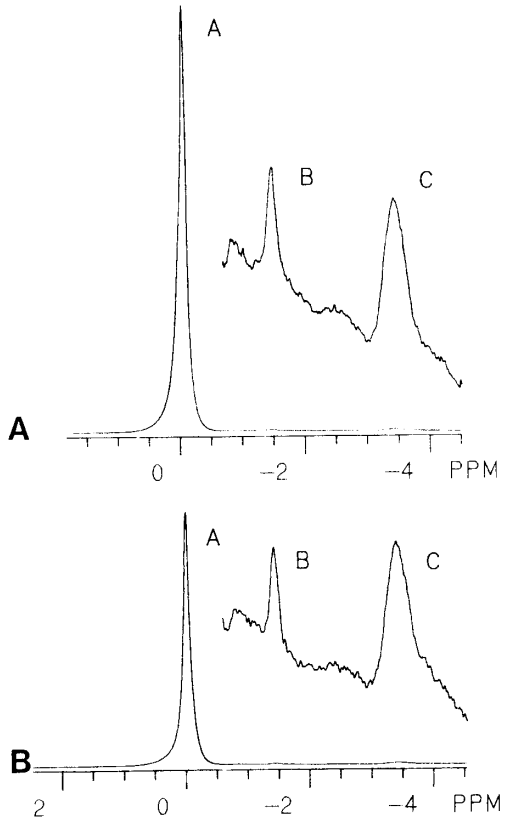

FIG. 1. Effect of preirradiation on ^1H NMR detected matabolites and water of the rabbit kidney *in vivo*. The rabbit kidney preparation and NMR probe design were previously described (*12*). The animal's temperature was maintained at 37°C. Each spectrum resulted from 16 averages of a $\pi/2$–τ–π–τ–acquire sequence in which τ = 50 ms. For this study the magnetic field strength was 4.7 T. Complex points (1K) were collected with an acquisition time of 256 ms. (A) Without preirradiation, interpulse delay = 6 s. (B) With preirradiation (0.4 W, 10 kHz below the ^1H_f resonance frequency) during the last 4 s of the interpulse delay. Peak assignments are (A) water, (B) trimethylamines, and (C) lipids.

doped water samples (to mimic *in vivo* T_2 values), or rabbit urine samples (not shown). The bandwidth (in Hz) of this effect was found to be independent of magnetic field strength (4.7 and 2 T) in canine heart muscle *in vitro* (Fig. 2C) and was equivalent to that found in 3% agar solutions (not shown). The field independence of the linewidth suggests that the restricted motion of this ^1H_r pool approaches the rigid-lattice condition (correlation time $> 10^{-7}$ s^{-1}) (*13*).

The effect of nonspecific radiofrequency bleed over from an irradiation is usually controlled by irradiating frequencies on either side of a specific resonance to look for frequency-specific effects (*8–10*). Since the ^1H_r component is symmetrical around ^1H_f (Figs. 2B and 2C) such controls are not possible in these experiments. However, the radiofrequency bleed from the off-resonance irradiation can be determined using the equation (*14*)

$$M_s = M_0(1 + T_{2f}^2\omega^2)/(1 + T_{2f}^2\omega^2 + \gamma^2 H_1^2 T_{1f} T_{2f}), \quad [1]$$

where ω is the offset frequency (hertz), γ is the gyromagnetic ratio, and H_1 is the magnetic field (tesla) induced by the irradiation. For this calculation, it is assumed

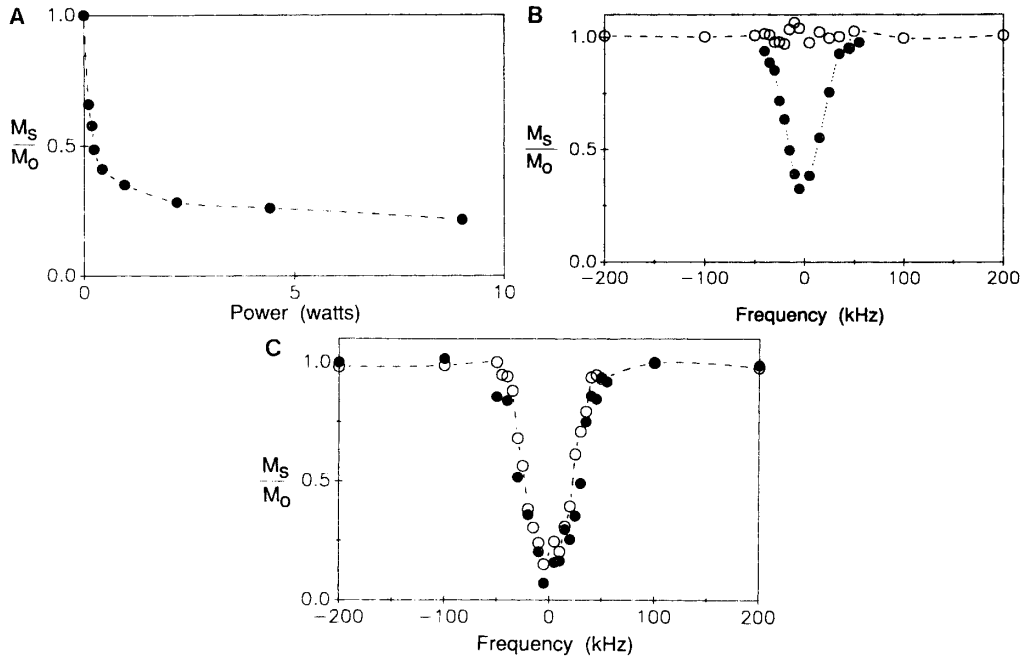

FIG. 2. (A) Effect of preirradiation power on the magnitude of the water resonance of the rabbit kidney *in vivo*. Preparation was identical to that used in Fig. 1. Spectra were acquired from a $\pi/8$ pulse that followed a 6-s interpulse delay where during the last 4 s there was rf preirradiation 5 kHz below the ^1H_f resonance frequency. Field strength was 4.7 T. (B) Effect of preirradiation frequency on the magnitude of the ^1H_f resonance from the rabbit kidney *in vivo* (●) and distilled water (○). Spectra were collected with a $\pi/8$–acquire pulse sequence with no echo in this case. Preirradiation power was 0.4 W. Field strength was 4.7 T. The *in vivo* kidney was the same preparation as that described in Fig. 1. The distilled water sample (25°C) in a glass tube was placed in the same coil as that used to collect the kidney data. The ^1H_f resonance frequency is at 0 Hz. Only data collected with the irradiation > 5 kHz off of the ^1H_f resonance are reported to minimize the effects of radiofrequency "bleed over." (C) Effect of preirradiation frequency on the magnitude of the ^1H_f resonance of canine cardiac tissue at two field strengths. Approximately 2 g of excised canine left ventricle muscle was placed in a glass tube and inserted into an NMR probe which could be tuned to the Larmor frequency of ^1H at the two fields. The temperature of these samples was 25°C. Spectra were collected as described in (A). (●) *Ex vivo* myocardium at 2.0 T. (○) Same tissue at 4.7 T.

that the irradiation time is on the order of T_{1f}. Other terms are defined in the text. The H_1 fields were estimated by determining the time required to produce a 360° flip by the decoupler at the power levels used in this study. The H_1 field varied from 7.0×10^{-6} T (0.4 W) to 3.5×10^{-5} T (8 W) in the kidney coil and 6×10^{-6} T (0.75 W) to 1.6×10^{-5} T (4.4 W) in the leg coil. Using an irradiation strength of 7×10^{-6} T at 5 kHz (which was used for the rate constant calculations in this study), a T_{2f} of 60 ms determined from spin-echo experiments, and a T_{1f} of 1.5 s (see Table 1), the bleed over of radiofrequency energy would result only in a <8% decrease in the ^1H_f resonance of the kidney. Thus, the irradiation effects observed in this study can be only minimally influenced by radiofrequency bleed over. The T_2 of ^1H_r (T_{2r}) can be estimated from the frequency plot shown in Figs. 2B and 2C using Eq. [1] which can be used to define the frequency response of the irradiation as a function of power, T_1 and T_2. The irradiation H_1 field was 7×10^{-6} T and a T_{1r} of 1 s was assumed (on the

TABLE 1

Effect of ^1H_r Saturation *in Vivo*

Tissue	T_{1fobs} (s)	T_{1fsat} (s)	M_s/M_0	T_{1f} (s)	k_a (s^{-1})	N
Kidney	1.5 ± 0.05	0.62 ± 0.08	0.30 ± 0.03	2.0 ± 0.2	1.2 ± 0.2	4
Skeletal muscle	1.5 ± 0.02	0.30 ± 0.03	0.07 ± 0.005	4.5 ± 0.5	3.1 ± 0.3	4

Note. All experiments were conducted on rabbits *in vivo* at 4.7 T and 37°C. T_{1fobs} and T_{1fsat} were determined using an inversion recovery experiment as previously described for ^{31}P in skeletal muscle (10) to obtain uniform times of saturation. For the kidney irradiation, 0.4 W was used to obtain saturation for the T_{1fsat} and M_s/M_0 determinations. For skeletal muscle irradiation, 1.6 W was used. All terms are defined in the text. T_{1f} and k_a were calculated using Eqs. [2] and [3], respectively. All data are presented as means ± standard error of the mean.

basis of the estimated correlation time of this dipolar spin). This resulted in a calculated T_{2r} of 0.2 ms assuming that the H_1 field was uniform as a function of frequency with these rather low Q (~90) loaded coils.

The T_1 of the ^1H_f pool in the absence of exchange (T_{1f}) can be determined from the effect of ^1H_r irradiation on the observed T_1 of the ^1H_f pool (T_{1sat}) and the steady-state ^1H_f magnetization before (M_0) and after (M_s) irradiation according to the following equation for an inversion recovery experiment (*11*),

$$T_{1f} = M_0/M_s \times T_{1sat}, \quad [2]$$

where M_0 is the ^1H_f steady-state magnetization without irradiation and M_s is with irradiation of ^1H_r. It should be noted that T_{1f} will still be influenced by other T_1 relaxation mechanisms, such as exchange with ^1H pools not irradiated and/or paramagnetic relaxation. To evaluate the effect of ^1H_r irradiation on the observed T_1 of ^1H_f, inversion recovery experiments were conducted in the presence and absence of irradiation of the ^1H_r pool. With a 0.4-W irradiation 5 kHz from the ^1H_f resonance, the T_1 changed from ~1.5 s without irradiation to ~0.62 s. This observed decrease in the T_1 of ^1H_f with irradiation of ^1H_r is also consistent with the exchange of magnetization between these two pools. Using Eq. [2], the T_{1f} values of whole kidney and rabbit skeletal muscle were calculated and are presented in Table 1. The T_{1f} of the kidney (~2 s) is much shorter than that of skeletal muscle (~4.5 s) implying that a T_1 relaxation mechanism other than magnetization exchange with the ^1H_r pool is affecting the ^1H_f pool in kidney. Likely candidates for this additional relaxation effect in kidney are paramagnetic metals, such as manganese, which are 10 times higher in concentration in kidney than in skeletal muscle (*15*).

The apparent psuedo-first-order rate constant for the movement of magnetization from ^1H_f to ^1H_r can be estimated from the effects of irradiating ^1H_r on the ^1H_f pool T_1 and steady-state magnetization using the equation (*7–10*)

$$k_a = 1/T_{1sat}[1 - M_s/M_0)], \quad [3]$$

where k_a is the apparent pseudo-first-order rate constant of magnetization transfer from ^1H_f to ^1H_r. Table 1 lists the calculated k_a values for rabbit skeletal muscle and

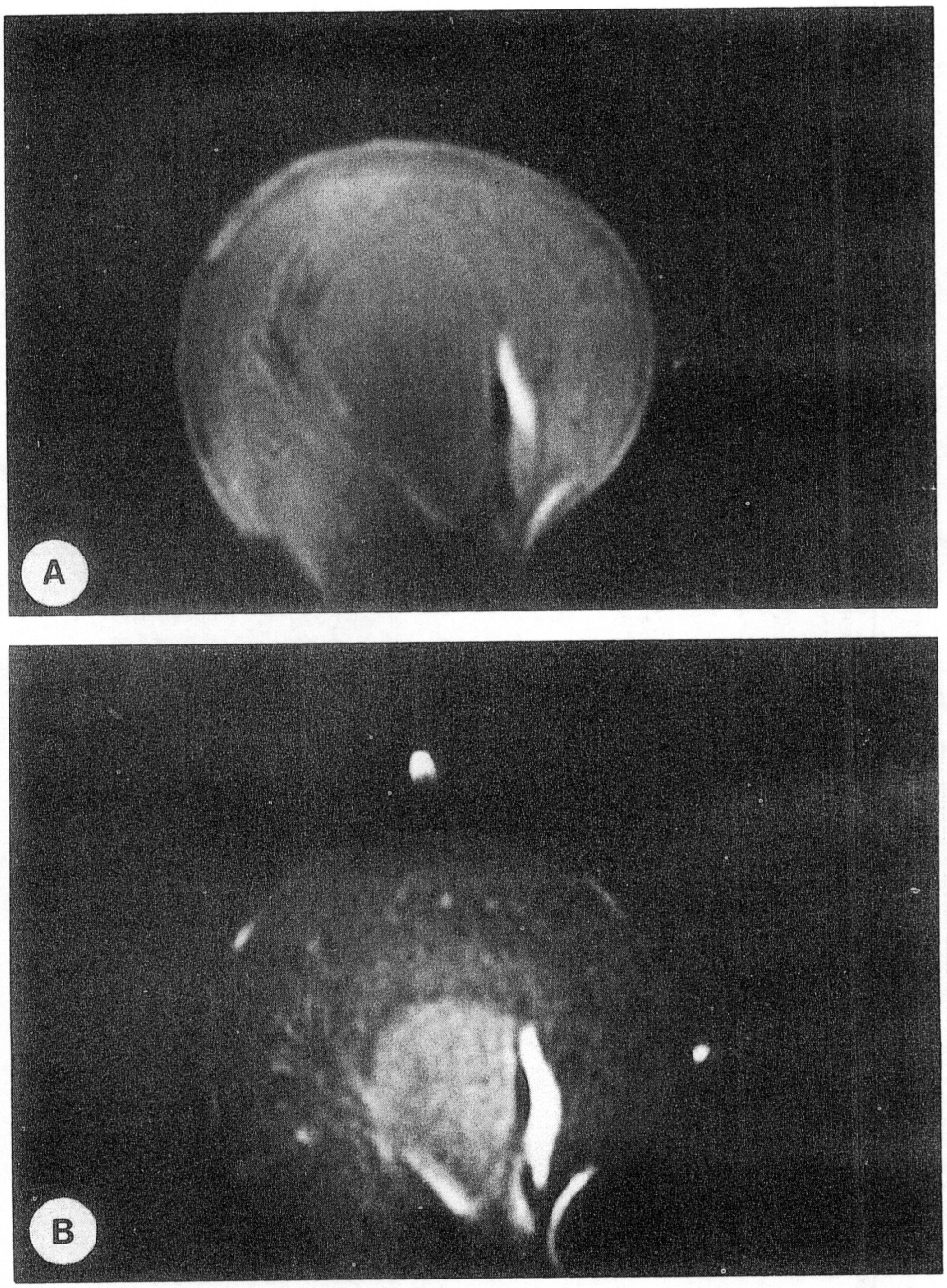

FIG. 3. Transverse spin-warp ^{1}H NMR images of rabbit kidney *in vivo*. The rabbit kidney preparation was as previously described (*12*). (A) ^{1}H density image, TR = 4.2 s, TE = 20 ms, field of view = 40 × 40 × 3 mm, digital matrix size 128 × 128. (B) Image collected using the same parameters as above, but with 3.5 s of 0.4 W irradiation 5 kHz below the resonance frequency of ^{1}H occurring just before slice selection. (C) Ratio image of image (B) divided by image (A) (M_s/M_0 image). (D) T_2-weighted kidney image. Collection conditions are the same as those in (A) only an 80-ms TE is used to generate T_2 contrast.

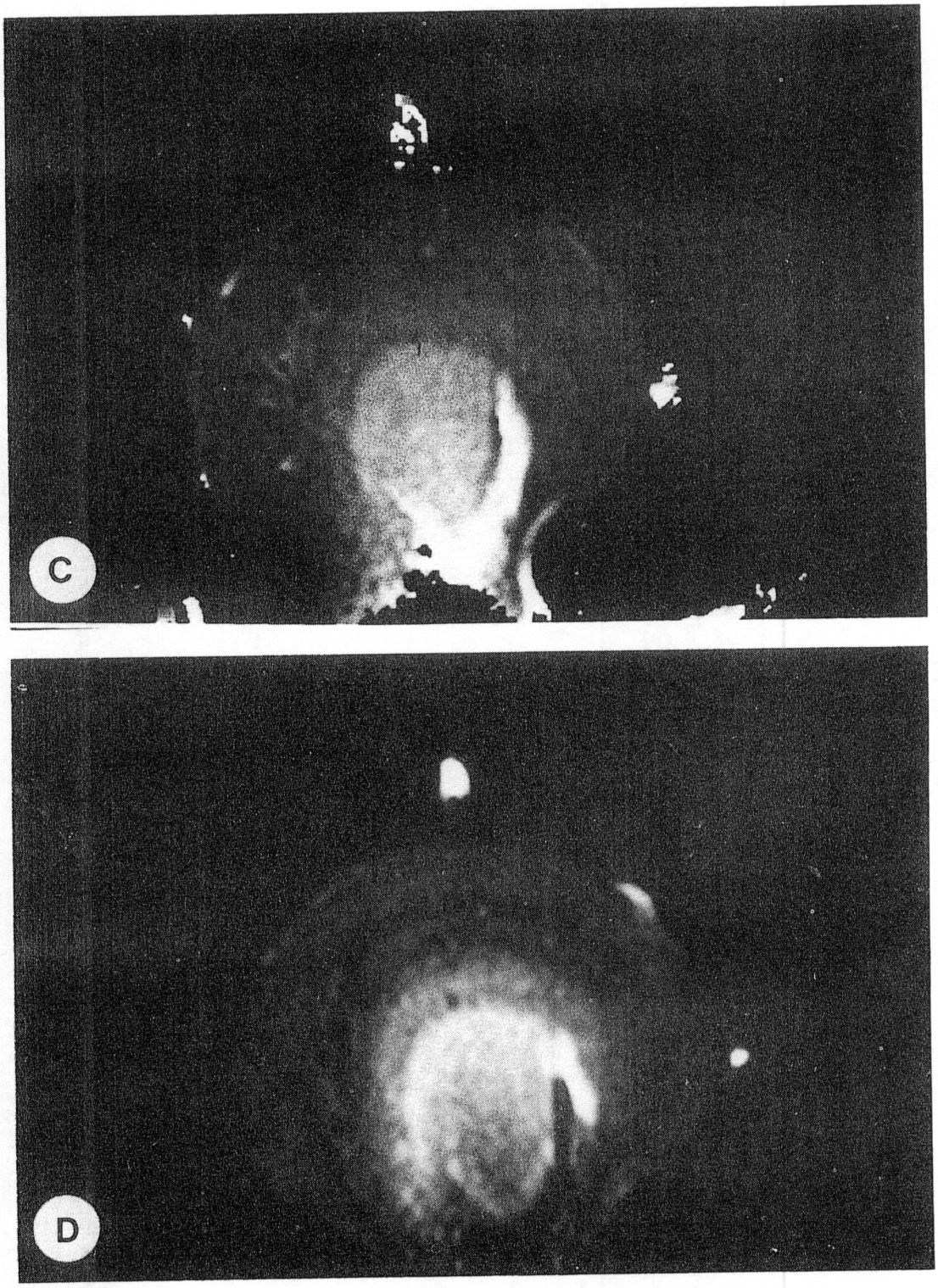

FIG. 3—*Continued*

kidney *in vivo* using Eq. [3]. The higher rate constant in skeletal muscle may be due to a higher protein content and/or a more efficient exchange mechanism in this tissue.

The values presented in Table 1 represent the average exchange occurring over the whole tissue studied. To investigate whether or not this magnetization exchange between ^1H_f and ^1H_r is homogeneous throughout tissues, the effect of ^1H_r irradiation on the distribution of steady-state ^1H magnetization was evaluated using NMR ^1H

imaging. Since the conventional NMR imaging experiment requires the collection of an echo, these techniques generally detect only the ^1H_f pool because of its relatively long T_2. NMR images of the kidney *in vivo* were taken using a conventional spin-warp sequence with and without irradiation 10 kHz from ^1H_f resonance. The images presented in Figs. 3A and 3B were collected with a 4.2-s recycle time (TR) and 20-ms echo time (TE) to minimize T_1 and T_2 contrast effects. Figure 3A is the control image without irradiation, Fig. 3B is with irradiation, and Fig. 3C is the ratio of irradiated image to the control image (i.e., an M_s/M_0 image (*9*)). To obtain an actual k_a image, the T_{1sat} of each voxel would have to be determined (*9*). The intensity of the renal cortex of the kidney was reduced by the irradiation to a much greater extent than the inner medulla resulting in improved contrast between these regions in the irradiated image (Fig. 3B) as well as the ratio image (Fig. 3C). This suggests that the exchange rate in the cortex of the kidney is higher than that in the inner medulla. The urine, blood vessels, and fat were not significantly affected by the irradiation resulting in a very high intensity signal from these structures in the irradiated (Fig. 3B) and ratio (Fig. 3C) image. The lack of effect on urine presumably is due to the limited number of macromolecules in urine resulting in limited magnetization exchange between a ^1H_r and a ^1H_f pool. Blood ^1H_f was less affected by irradiation since the flow of blood results in ^1H spins entering the field of view which were not irradiated if they came from a region outside of the coil. Furthermore, the irradiation of ^1H_r in whole rabbit blood (hematocrit 43%) *in vitro* resulted in a decrease in ^1H_f which was approximately twofold less than that of the whole kidney, indicating that the ^1H_f–^1H_r exchange is relatively slow in blood. The fat was unaffected since this resonance does not exchange with the ^1H_r component (Fig. 1). These results demonstrate that the magnitude of the irradiation effect on ^1H_f is tissue specific and causes tissue contrast in the NMR image on the basis of the exchange process as well as flow from regions not affected by the irradiation.

DISCUSSION

These data demonstrate that the ^1H_f pool is exchanging magnetization with an immobilized ^1H_r pool. On the basis of the present data it is not possible to establish what portion of this exchange is due to a chemical exchange of protons or a through space interaction with immobilized water and/or macromolecules. Preliminary studies ($n = 3$) with natural abundance or isotopically enriched ^2H water signals in rabbit skeletal muscle *in vivo* reveal that no saturation transfer is observed with a broad component using conditions similar to those outlined for ^1H. This result may imply that the dipolar cross-relaxation may be the dominate factor in the saturation transfer observed with ^1H. However, the quadrupolar relaxation of ^2H could result in such a broad line for the immobilized water that it could not be effectively saturated in these protocols and/or the relaxation processes could simply be dominated by the quadrupolar relaxation process. Clearly, more studies are required to establish the actual mechanism causing the saturation transfer observed between ^1H_f and ^1H_r.

Independent of the mechanism, the exchange between the ^1H_f and the ^1H_r pool will result in the observed ^1H_f relaxation times (i.e., both T_1 and T_2) to be affected by the relaxation times of the ^1H_r pool according to the equation

$$1/T_{\text{fobs}} = 1/T_{\text{f}} + X_{\text{r}}/(T_{\text{r}} + 1/k_{\text{r}}), \quad [4]$$

where T_{fobs} is the observed T_1 or T_2 of the ^1H_f pool, T_f is the T_1 or T_2 of the ^1H_f pool in the absence of exchange, X_r is the mole fraction of the ^1H_r pool, T_r is the T_1 or T_2 of the ^1H_r pool in the absence of exchange, and k_r is the rate constant of the transfer of magnetization from the ^1H_r to the ^1H_f pool. This equation is valid only when the two exchanging pools have the same chemical shift (see Fig. 2B) and X_r is less than 0.3 (*13*). With the ^1H_r pool effectively at the rigid lattice condition, the T_2 of this component will be much shorter than T_1 (*13*). Thus, according to Eq. [4], we might expect the effect of the magnetization exchange to be much more pronounced for the observed T_2 of the ^1H_f pool than for its T_1. Indeed, the M_s/M_0 images obtained for the rabbit kidney (Fig. 3C) and cat head (not shown) correlate with conventional T_2-weighted images (Fig. 3D) and not T_1-weighted images with regard to overall tissue contrast patterns. This latter result suggests that the pathway of relaxation through ^1H_r is very significant in determining the observed T_2 in these tissues.

The imaging technique described generates tissue contrast on the basis of the extent of magnetization transfer occurring in the tissue (i.e., magnetization transfer contrast (MTC)). This contrast is very similar, on a gross level, to T_2 contrast although it can be obtained without long echo times. Indeed, we have been successful in obtaining high-contrast images using gradient-recalled echo sequences (5-ms TE) using this approach (Wolff and Balaban, in preparation).

In summary, it has been demonstrated in a variety of biological tissues that the free water component of the ^1H NMR signal is in magnetization exchange with a relatively immobile component. The pseudo-first-order rate constant of this exchange varied from 0 to $\sim 4\ s^{-1}$ depending on the particular tissue or fluid type. The exchange mechanism could include chemical exchange and/or cross-relaxation processes between the free water ^1H and the ^1H in or associated with large cellular macromolecules (proteins, membranes, etc.). The magnitude of this exchange process was imaged in tissue and provided a unique quantitative form of contrast for NMR imaging. Since the image contrast generated using this method is specific to the exchange between ^1H_f and ^1H_r, this technique may prove valuable in diagnosis or characterization of cancer, edema, or other pathologies where the specific relaxation mechanism may be useful in determining the nature of the disease.

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