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Effects of Off-Resonance Irradiation, Cross-Relaxation, and Chemical Exchange on Steady-State Magnetization and Effective Spin–Lattice Relaxation Times

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In the presence of an off-resonance radiofrequency field, recovery of longitudinal magnetization to a steady state is not purely monoexponential. Under reasonable conditions with zero initial magnetization, recovery is nearly exponential and an effective relaxation rate constant $R_{1\mathrm{eff}} = 1/T_{1\mathrm{eff}}$ can be obtained. Exact and approximate formulas for $R_{1\mathrm{eff}}$ and steady-state magnetization are derived from the Bloch equations for spins undergoing cross-relaxation and chemical exchange between two sites in the presence of an off-resonance radiofrequency field. The relaxation formulas require that the magnetization of one spin is constant, but not necessarily zero, while the other spin relaxes. Extension to three sites with one radiofrequency field is explained. The special cases of off-resonance effects alone and with cross-relaxation or chemical exchange, cross-relaxation alone, and chemical exchange alone are compared. The inaccuracy in saturation transfer measurements of exchange rate constants by published formulas is discussed for the creatine kinase reaction. © 2000 Academic Press

Key Words: **Bloch equations; chemical exchange; off-resonance effects; saturation transfer; T_1 relaxation.**

INTRODUCTION

Many NMR experiments are conducted with a constant low-power radiofrequency (RF) irradiation to saturate one or more spins while observing another spin. Examples include decoupling experiments, cross-relaxation measurements including the nuclear Overhauser effect (NOE), and saturation-transfer measurements of chemical exchange rate constants. In this work we focus on saturation-transfer measurements of chemical exchange (*1–16*). For completeness, cross-relaxation is included, so the formulas can be applied to NOE measurements (*17, 18*) and magnetic resonance imaging (MRI) magnetization-transfer measurements (*19–22*). Homonuclear cross-relaxation is common in ^{1}H spin systems and usually is negligible in ^{31}P spectroscopy of biological systems.

In a saturation-transfer experiment the signal intensity of a detected spin D is measured with and without saturation of an exchanging spin E (Figs. 1A and 1B). The effective spin–lattice relaxation rate constant of spin D, $R_{1\mathrm{eff}} = 1/T_{1\mathrm{eff}}$, is measured while the exchanging spin E is saturated (*2–8*). An alternative method involves calculating the intrinsic R_1 of spin D in the absence of chemical exchange (*1, 9, 23*).

Off-resonance irradiation decreases the steady-state signal intensity of a resonance (*24, 25*), and the spin–spin relaxation rate constant $R_2 = 1/T_2$ can be measured from this signal decrease (*26, 27*). The decrease in intensity of spin D during saturation of spin E will therefore be caused by both direct off-resonance effects ("spillover") and chemical exchange. Attempts to correct for off-resonance effects involve using a control irradiation, the same distance from peak D but in the opposite direction (Fig. 1C). Usually the intensity of spin D during the control irradiation is used as the equilibrium magnetization in the formulas for determining the exchange rate constant (*1–4*). A recent correction factor has accounted for exchange of D spins with partially saturated E spins during the control irradiation (*9*). The $R_{1\mathrm{eff}}$ also changes with off-resonance irradiation (*14, 28*), and the new $T_{1\mathrm{eff}}$ is sometimes called the "spin–lattice relaxation time in the rotating frame with off-resonance irradiation," $T_{1\rho}^{\mathrm{off}}$ (*29, 30*). This increase in $R_{1\mathrm{eff}}$ usually has not been included in the formulas for calculating the exchange rate constants. Potts and co-workers suggested a formula to include the increased $R_{1\mathrm{eff}}$, but the theoretical justification for the method was not specified (*14*). Furthermore, the method requires measuring intensities and relaxation rates in the presence of the RF field without chemical exchange.

The purpose of the present work is to investigate the relationship between the signal intensity decrease and the $R_{1\mathrm{eff}}$ increase with off-resonance irradiation, as well as the effect of chemical exchange and cross-relaxation on these measurements. After obtaining a complete solution of the modified Bloch equations with constant spin E magnetization, approximations are introduced to simplify some formulas. Special cases are then considered, including off-resonance effects alone, cross-relaxation with and without off-resonance effects, and chemical exchange with and without off-resonance effects. Finally, inaccuracies in saturation-transfer measurements of exchange rate constants with published formulas are discussed.

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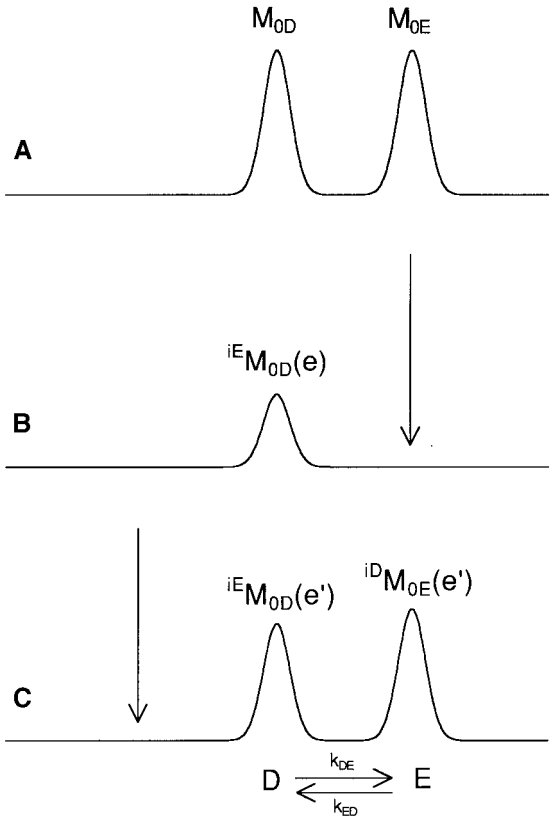

FIG. 1. Saturation-transfer experiment for a two-spin system, a detected spin D, and an exchanging spin E. (A) No irradiation, for measurement of the equilibrium values M_{0D} and M_{0E}. (B) Spin E saturated, for measurement of ${}^{iE}M_{0D}(e)$. The intensity of spin D is decreased by chemical exchange and perhaps by direct off-resonance effects. (C) The usual control experiment, with the irradiation moved from E to the opposite side of D, for measurement of ${}^{iE}M_{0D}(e')$ and ${}^{iD}M_{0E}(e')$. The intensity of each spin may be decreased by direct off-resonance effects, and by exchange with the other partially saturated spin.

Extension to three sites with one radiofrequency field is discussed in Appendix A.

BLOCH EQUATIONS WITH CHEMICAL EXCHANGE AND CROSS-RELAXATION

The Modified Bloch Equations

In general the steady-state magnetizations with off-resonance irradiation will be different from thermal equilibrium, and this difference must be indicated. The notation for magnetization levels and relaxation rate constants is summarized in Table 1. All relaxation rates refer to spin D unless otherwise indicated. Magnetization levels will include a subscript D or E to indicate the relevant spin. A preceding superscript $E(D)$ indicates chemical exchange with spin $E(D)$, σ indicates cross-relaxation, and i indicates direct off-resonance effects of the irradiation. Direct irradiation of spin E will be indicated by (e), and irradiation of a control position (see Fig. 1C) will be indicated by (e'). For example, ${}^{E}R_1$ is the $R_{1\text{eff}}$ of spin D exchanging with spin E, ${}^{i}R_1$ is $R_{1\text{eff}}$ of spin D with direct off-resonance effects but without chemical exchange or cross-relaxation, and ${}^{i\sigma E}M_{0D}(e)$ is the steady-state longitudinal magnetization of spin D while spin E is irradiated with off-resonance effects, chemical exchange, and cross-relaxation included. Magnetization components normalized to equilibrium magnetization will be represented by $E_x = M_{xE}/M_{0E}$, $E_y = M_{yE}/M_{0E}$, and $E_z = M_{zE}/M_{0E}$, with similar relationships for spin D.

We will assume that the magnetizations can be described accurately by the Bloch equations (*24*) modified for chemical exchange (*5–10, 13, 23, 31, 32*) and cross-relaxation (*18–22, 31*). The modified equations in the reference frame rotating at the RF frequency, with the $\mathbf{B}_1$ field along the $+x$ axis, are (*33*)

$$dM_{zD}/dt = -\omega_1 M_{yD} + R_1(M_{0D} - M_{zD}) - (k_{DE} + \sigma_{DE})M_{zD} + (k_{ED} + \sigma_{ED})M_{zE} \quad [1]$$

$$dM_{xD}/dt = \omega M_{yD} - R_2 M_{xD} - k_{DE}M_{xD} + k_{ED}M_{xE} \quad [2]$$

$$dM_{yD}/dt = -\omega M_{xD} + \omega_1 M_{zD} - R_2 M_{yD} - k_{DE}M_{yD} + k_{ED}M_{yE}, \quad [3]$$

where $\omega_1 = \gamma B_1$ is the precession frequency about the RF field, $\omega = \omega_0(D) - \omega_{\text{rf}}$ is the difference between the resonance frequency of spin D and the RF frequency (Fig. 1), k_{DE} and k_{ED} are the chemical-exchange rate constants, and σ_{DE} and σ_{ED} are the cross-relaxation rate constants. The rate constant R_1 is the spin–lattice relaxation rate constant in the absence of any magnetization exchange. There are also three similar equations for spin E. Transverse magnetization precesses from $+x$ to $-y$ for $\omega > 0$, and an RF pulse on the $+x$ axis moves a magnetization vector from $+z$ to $+y$. The cross-relaxation rate constant σ_{DE} may be positive or negative; a positive σ_{DE} results in a signal loss when spin E is saturated. This is the opposite of the standard convention for NOE experiments. The calculations assume that possible complicating factors such as spin–spin coupling are absent. At equilibrium there is no net exchange of magnetization, so

$$k_{ED}M_{0E} = k_{DE}M_{0D} \quad [4]$$

$$\sigma_{ED}M_{0E} = \sigma_{DE}M_{0D}. \quad [5]$$

These relationships, sometimes referred to as "detailed balance," allow Eqs. [1]–[3] to be converted into other forms more commonly used for cross-relaxation (*18–22, 31*). Rearrangement of Eqs. [1]–[3] and application of Eqs. [4] and [5] to Eq. [1] yields

$$dM_{zD}/dt = -\omega_1 M_{yD} + {}^{\sigma E}R_1({}^{\sigma E}M_{0D} - M_{zD}) \quad [6]$$

$$dM_{xD}/dt = \omega M_{yD} - {}^{E}R_2 M_{xD} + k_{ED}M_{xE} \quad [7]$$

TABLE 1
Notation for Magnetization in the Presence of Chemical Exchange, Cross-Relaxation, and Off-Resonance Effects

Symbol	Meaning
Preceding superscripts	
i	Includes effects of off-resonance irradiation
σ	Cross-relaxation included
E	Chemical exchange with spin E, with constant spin E magnetization
Magnetization symbols	
M_{0D}, M_{0E}	Equilibrium magnetization with no irradiation
${}^{E}M_{0D}$	M_{zD}(ss) with chemical exchange with spin E, Eq. [11]
${}^{\mathrm{i}}M_{0D}$	M_{zD}(ss) with off-resonance irradiation
${}^{\sigma}M_{0D}$	M_{zD}(ss) with cross-relaxation, Eq. [11]
(e)	Spin E is irradiated (saturated), Fig. 1B
(e')	Irradiation of a control position for spin E, Fig. 1C
${}^{\mathrm{i}E}M_{0D}(e)$	M_{zD}(ss) with spin E irradiated, including off-resonance effects
${}^{\sigma E}M_{0D}$	M_{zD}(ss) with chemical exchange and cross-relaxation, Eq. [11]
${}^{\mathrm{i}\sigma E}M_{0D}$	M_{zD}(ss) with chemical exchange, cross-relaxation, and off-resonance effects, Eq. [26]
Magnetization ratios	
D_x, D_y, D_z	M_{xD}/M_{0D}, M_{yD}/M_{0D}, M_{zD}/M_{0D}
E_x, E_y, E_z	M_{xE}/M_{0E}, M_{yE}/M_{0E}, M_{zE}/M_{0E}
${}^{\mathrm{i}E}D_z(e')$	${}^{\mathrm{i}E}M_{zD}(e')/M_{0D}$
${}^{\mathrm{i}E}D_z(e)$	${}^{\mathrm{i}E}M_{zD}(e)/M_{0D}$
${}^{\mathrm{i}D}E_z(e')$	${}^{\mathrm{i}D}M_{zE}(e')/M_{0E}$
${}^{\mathrm{i}D}E_z(e)$	${}^{\mathrm{i}D}M_{zE}(e)/M_{0E}$
Relaxation rate constants	
α_i	Real or complex time constants, roots of a cubic equation in the Laplace transform
${}^{\mathrm{i}}R_1$	$R_{1\mathrm{eff}}$ with off-resonance effects ($= -\alpha_1$)
${}^{\mathrm{i}\sigma E}R_1$	$R_{1\mathrm{eff}}$ with chemical exchange, cross-relaxation, and off-resonance effects

Note. M_{zD}(ss) is steady-state longitudinal magnetization of spin D.

$$dM_{yD}/dt = -\omega M_{xD} + \omega_1 M_{zD} - {}^{E}R_2 M_{yD} + k_{ED} M_{yE}, \quad [8]$$

where

$$^{\sigma E}R_1 = {}^{\sigma E}R_1(e) = R_1 + k_{DE} + \sigma_{DE} \quad [9]$$

$$^{E}R_2 = {}^{E}R_2(e) = R_2 + k_{DE} \quad [10]$$

$$^{\sigma E}M_{0D} = M_{0D}[R_1 + (k_{DE} + \sigma_{DE}) E_z]/{}^{\sigma E}R_1. \quad [11]$$

Notice that steady-state longitudinal magnetization ${}^{\sigma E}M_{0D}$ depends on spin E magnetization and cross-relaxation, while ${}^{\sigma E}R_1$ depends on cross-relaxation but not spin E magnetization.

The General Solution with Constant Spin E Magnetization

The Bloch equations have been solved by application of a Laplace transform (*22, 25, 33, 34*) and by a multiple-derivative method (*35, 36*). The Laplace transform converts the differential equations into algebraic equations, which can be solved with standard techniques. The inverse Laplace transform then yields the final solution. Appendix B contains corrections of some errors in these and other previous publications. An analytic solution with chemical exchange in the absence of an RF field and cross-relaxation has also been presented (*37, 38*).

No complete analytic solution is available for the general equations [6]–[8] plus the three corresponding equations for spin E, with time-dependent changes in M_{xD}, M_{yD}, M_{zD}, M_{xE}, M_{yE}, and M_{zE}. One approach is to use specific values for the relaxation and exchange rate constants and the relative equilibrium magnetizations and obtain a numerical solution of the Bloch equations (*2*). Another solution involves finding the roots of a sixth-order polynomial numerically and inserting these values into an analytic solution. This method has been applied to the case of cross-relaxation without chemical exchange (*22*). With these numerical solutions it is not easy to determine how a change in one parameter affects the final result.

Most saturation-transfer formulas achieve an analytic solution by assuming $M_{xE} = M_{yE} = M_{zE} = 0$. A useful compromise is to assume that M_{xE}, M_{yE}, and M_{zE} are held at constant values. This is clearly true for steady-state conditions, so the formulas for the steady-state magnetization ${}^{\mathrm{i}\sigma E}M_{0D}$ will be valid when the intensity of spin E is reduced by off-resonance effects or by exchange with another saturated spin. Furthermore, this assumption is nearly true for a strong on-resonance saturating pulse ($\omega_1 \gg R_2$), so experimental measurements of ${}^{\mathrm{i}\sigma E}R_1$ will be good estimates if spin E is nearly saturated.

The following derivation based on the Laplace transform is similar to the derivation presented by Mulkern and Wil-

liams for the case with no chemical exchange or cross-relaxation (*33*). Equations [6]–[8] are in the same form as their equations, and we have used their notation in many cases. The reader is referred to that work for the detailed steps. The solution to the Bloch equations is equal to the sum of the residues of the transformed equations times $\exp(\alpha_i t)$, where $\alpha_0 = 0$ and α_1, α_2, and α_3 are the three roots of a cubic equation. Thus the solution for each component of magnetization is of the form

$$M_j(t) = M_j(\mathrm{ss}) + M_{j1}\exp(\alpha_1 t) + M_{j2}\exp(\alpha_2 t) + M_{j3}\exp(\alpha_3 t), \quad [12]$$

where $j = x$, y, or z.

If M_{xE}, M_{yE}, and M_{zE} are constant, the steady-state values for M_{xD}, M_{yD}, and M_{zD} can be determined by setting $dM_{xD}/dt = dM_{yD}/dt = dM_{zD}/dt = 0$, or from the $\alpha = 0$ pole of the Laplace transform (*33*). The results presented below are similar to the well-known results without chemical exchange (*24, 25, 33–36, 39*) plus new terms involving M_{xE} and M_{yE}, and with R_1, R_2, and M_{0D} replaced by ${}^{\sigma E}R_1$, ${}^{E}R_2$, and ${}^{\sigma E}M_{0D}$ from Eqs. [9]–[11].

For the time-dependent evolution of magnetization, application of the Laplace transform to Eqs. [6]–[8] with constant spin E magnetization yields the same equations as those published previously without chemical exchange (*33*) with the substitutions of Eqs. [9]–[11] and with $\alpha_i f_0$ and $\alpha_i g_0$ replaced by $\alpha_i^E f_0$ and $\alpha_i^E g_0$, where

$$\alpha_i^E f_0 = \alpha_i f_0 + k_{ED} M_{xE} \quad [13]$$

$$\alpha_i^E g_0 = \alpha_i g_0 + k_{ED} M_{yE}. \quad [14]$$

In these and other expressions f_0, g_0, and m_0 are the initial x, y, and z magnetizations. One time constant α_1 is real (exponential decay), and the other two may be complex conjugates (damped oscillations) or real:

$$\alpha_1 = -(2\,{}^{E}R_2 + {}^{\sigma E}R_1)/3 + A + B \quad [15]$$

$$\alpha_2 = -(2\,{}^{E}R_2 + {}^{\sigma E}R_1)/3 - (A + B)/2 + i3^{1/2}(A - B)/2 \quad [16]$$

$$\alpha_3 = -(2\,{}^{E}R_2 + {}^{\sigma E}R_1)/3 - (A + B)/2 - i3^{1/2}(A - B)/2, \quad [17]$$

where

$$A = (-b/2 + c^{1/2})^{1/3} \quad [18]$$

$$B = (-b/2 - c^{1/2})^{1/3} \quad [19]$$

$$a = [3(\omega^2 + \omega_1^2) - ({}^{\sigma E}R_1 - {}^{E}R_2)^2]/3 \quad [20]$$

$$b = ({}^{\sigma E}R_1 - {}^{E}R_2)[2({}^{\sigma E}R_1 - {}^{E}R_2)^2 + 18\omega^2 - 9\omega_1^2]/27 \quad [21]$$

$$c = b^2/4 + a^3/27 = \{(\omega^2 + \omega_1^2)^3 + ({}^{\sigma E}R_1 - {}^{E}R_2)^2[\omega^2(2\omega^2 + \{{}^{\sigma E}R_1 - {}^{E}R_2\}^2) - \omega_1^2(\omega_1^2/4 + 5\omega^2)]\}/27. \quad [22]$$

Notice that $\alpha_i^E f_0$ and $\alpha_i^E g_0$ depend on spin E magnetization while the three α_i do not. Equations [15]–[17] require that the cube roots A and B be chosen so that $AB = -a/3$. This is satisfied if A and B are real for $c > 0$ and if they are complex conjugates $B = A^*$ for $c < 0$. For more information on the cubic equation whose roots are the three α_i, see (*33*). The solution of cubic equations is available in several books of mathematical tables and at the internet sites http://www.ping.be/math and http://history.math.csusb.edu/HistTopics/Quadratic_etc_equations.html#47.

The complete solution is shown in the following equations. Notice that longitudinal and transverse magnetizations decay or oscillate with the same three rate constants α_i, although the coefficients of these three components (m_i, f_i, g_i) are different for the x, y, and z magnetizations.

$${}^{i\sigma E}M_{zD}(t) = {}^{i\sigma E}M_{0D} + m_1\exp(\alpha_1 t) + m_2\exp(\alpha_2 t) + m_3\exp(\alpha_3 t) \quad [23]$$

$${}^{i\sigma E}M_{xD}(t) = {}^{i\sigma E}M_{xD}(\mathrm{ss}) + f_1\exp(\alpha_1 t) + f_2\exp(\alpha_2 t) + f_3\exp(\alpha_3 t) \quad [24]$$

$${}^{i\sigma E}M_{yD}(t) = {}^{i\sigma E}M_{yD}(\mathrm{ss}) + g_1\exp(\alpha_1 t) + g_2\exp(\alpha_2 t) + g_3\exp(\alpha_3 t), \quad [25]$$

where

$${}^{i\sigma E}M_{0D} = \frac{{}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1(\omega^2 + {}^{E}R_2^2) + k_{ED}\omega_1(\omega M_{xE} - {}^{E}R_2 M_{yE})}{{}^{\sigma E}R_1(\omega^2 + {}^{E}R_2^2) + {}^{E}R_2\omega_1^2} \quad [26]$$

$$m_1 = \frac{[({}^{E}R_2 + \alpha_1)^2 + \omega^2]({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_1) - \omega_1\alpha_1[{}^{E}g_0({}^{E}R_2 + \alpha_1) - \omega\,{}^{E}f_0]}{\alpha_1(\alpha_1 - \alpha_2)(\alpha_1 - \alpha_3)} \quad [27]$$

$$m_2 = \frac{[({}^{E}R_2 + \alpha_2)^2 + \omega^2]({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_2) - \omega_1\alpha_2[{}^{E}g_0({}^{E}R_2 + \alpha_2) - \omega\,{}^{E}f_0]}{\alpha_2(\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)} \quad [28]$$

$$m_3 = \frac{[({}^{E}R_2 + \alpha_3)^2 + \omega^2]({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_3) - \omega_1\alpha_3[{}^{E}g_0({}^{E}R_2 + \alpha_3) - \omega\,{}^{E}f_0]}{\alpha_3(\alpha_3 - \alpha_1)(\alpha_3 - \alpha_2)} \quad [29]$$

$${}^{i\sigma E}M_{xD}(\mathrm{ss}) = \frac{{}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1\omega\omega_1 + k_{ED}[(\omega_1^2 + {}^{\sigma E}R_1\,{}^{E}R_2) \times M_{xE} + \omega\,{}^{\sigma E}R_1 M_{yE}]}{{}^{\sigma E}R_1(\omega^2 + {}^{E}R_2^2) + {}^{E}R_2\omega_1^2} \quad [30]$$

$$f_1 = \frac{\omega\omega_1({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_1) + \alpha_1\,{}^{E}g_0\omega({}^{\sigma E}R_1 + \alpha_1) + \alpha_1\,{}^{E}f_0[({}^{E}R_2 + \alpha_1)({}^{\sigma E}R_1 + \alpha_1) + \omega_1^2]}{\alpha_1(\alpha_1 - \alpha_2)(\alpha_1 - \alpha_3)} \quad [31]$$

$$f_2 = \frac{\omega\omega_1({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_2) + \alpha_2\,{}^{E}g_0\omega({}^{\sigma E}R_1 + \alpha_2) + \alpha_2\,{}^{E}f_0[({}^{E}R_2 + \alpha_2)({}^{\sigma E}R_1 + \alpha_2) + \omega_1^2]}{\alpha_2(\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)} \quad [32]$$

$$f_3 = \frac{\omega\omega_1({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_3) + \alpha_3\,{}^{E}g_0\omega({}^{\sigma E}R_1 + \alpha_3) + \alpha_3\,{}^{E}f_0[({}^{E}R_2 + \alpha_3)({}^{\sigma E}R_1 + \alpha_3) + \omega_1^2]}{\alpha_3(\alpha_3 - \alpha_1)(\alpha_3 - \alpha_2)} \quad [33]$$

$${}^{i\sigma E}M_{yD}(\mathrm{ss}) = \frac{{}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1\,{}^{E}R_2\omega_1 + k_{ED}\,{}^{\sigma E}R_1[{}^{E}R_2 M_{yE} - \omega M_{xE}]}{{}^{\sigma E}R_1(\omega^2 + {}^{E}R_2^2) + {}^{E}R_2\omega_1^2} \quad [34]$$

$$g_1 = \frac{\omega_1({}^{E}R_2 + \alpha_1)({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_1) + \alpha_1({}^{\sigma E}R_1 + \alpha_1) \times [{}^{E}g_0({}^{E}R_2 + \alpha_1) - \omega\,{}^{E}f_0]}{\alpha_1(\alpha_1 - \alpha_2)(\alpha_1 - \alpha_3)} \quad [35]$$

$$g_2 = \frac{\omega_1({}^{E}R_2 + \alpha_2)({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_2) + \alpha_2({}^{\sigma E}R_1 + \alpha_2) \times [{}^{E}g_0({}^{E}R_2 + \alpha_2) - \omega\,{}^{E}f_0]}{\alpha_2(\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)} \quad [36]$$

$$g_3 = \frac{\omega_1({}^{E}R_2 + \alpha_3)({}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1 + m_0\alpha_3) + \alpha_3({}^{\sigma E}R_1 + \alpha_3) \times [{}^{E}g_0({}^{E}R_2 + \alpha_3) - \omega\,{}^{E}f_0]}{\alpha_3(\alpha_3 - \alpha_1)(\alpha_3 - \alpha_2)}. \quad [37]$$

Working with These Equations

The preceding equations show the complete solution and can be used for individual calculations by inserting values for the relaxation and exchange rate constants. However, it is not easy to discern relative rates and intensities. In certain cases some general conclusions can be drawn and some approximations can simplify the formulas.

TABLE 2
Maximum Percentage of Error in Estimates with Approximate Formulas

		R_2 (s^{-1})		
Variable	Eq.	2	10	100
$A + B$	49	0.0003	0.020	2.28
$A - B$	50	<0.0001	0.0060	0.71
AB	51	0.0004	0.027	3.3
α_1	53	<0.0001	0.024	6.6
$\mathrm{Re}(\alpha_2)$	54	<0.0001	0.0043	0.56
$\mathrm{Im}(\alpha_2)$	56	<0.0001	0.0060	0.71
d_2	57	0.0024	0.087	8.7
$\lvert m_2\rvert$	60	0.0030	0.13	14.6
d_1	62	0.0010	0.075	6.4
m_1	63	0.0032	0.082	8.0
${}^{i}M_{0D}$	69	0.0034	0.10	8.9

Note. The differences between the actual and approximate values (calculated with the equations shown in the second column) were calculated for each R_2 with ω = 320, 960, 2880, and 8640 rad/s and ω_1 = 20, 60, 180, and 540 rad/s, all with $R_1 = 1\ s^{-1}$. The largest of the 16 differences is shown as a percentage of the actual value. With $R_2 = 1\ s^{-1}$, all differences were <0.0008%.

Under certain conditions (at or near resonance with ${}^{E}R_2 - {}^{\sigma E}R_1 > \omega_1$)$c < 0$, so A = B* and all three α_i are real, leading to triexponential relaxation (*33*, *40*). In most instances, including typical saturation-transfer measurements, $c > 0$, so α_1 is real while $\alpha_2 = \alpha_3^*$, $m_2 = m_3^*$, $f_2 = f_3^*$, and $g_2 = g_3^*$. In this case $\alpha_1 = -{}^{i\sigma E}R_1$ corresponds to monoexponential relaxation, while α_2 and α_3 describe a damped oscillation (*33*, *35*, *36*). For this common case Eq. [23] becomes

$${}^{i\sigma E}M_{zD}(t) = {}^{i\sigma E}M_{0D} + m_1\exp(\alpha_1 t) + 2|m_2|\exp[\mathrm{Re}(\alpha_2)t]\cos[\mathrm{Im}(\alpha_2)t + \phi], \quad [38]$$

where $\sin\phi = \mathrm{Im}(m_2)/|m_2|$ and $\cos\phi = \mathrm{Re}(m_2)/|m_2|$. The angle ϕ can be written as $\arctan[\mathrm{Im}(m_2)/\mathrm{Re}(m_2)]$ as long as care is taken to choose the correct quadrant for the angle, because the arctangent normally is defined over the range $-\pi/2$ to $\pi/2$. Similar equations apply to ${}^{i\sigma E}M_{xD}(t)$ and ${}^{i\sigma E}M_{yD}(t)$, with ${}^{i\sigma E}M_{0D}$, m_1, and m_2 replaced by ${}^{i\sigma E}M_{xD}(\mathrm{ss})$, f_1, and f_2 or by ${}^{i\sigma E}M_{yD}(\mathrm{ss})$, g_1, and g_2. Thus $-\alpha_1$ corresponds to the effective spin–lattice relaxation rate $R_{1\mathrm{eff}}$.

Approximations

The time-dependent solution for longitudinal magnetization in Eq. [38] requires knowledge of α_1, $\mathrm{Re}(\alpha_2)$, $\mathrm{Im}(\alpha_2)$, $\mathrm{Re}(m_2)$, $\mathrm{Im}(m_2)$, and m_1. Approximate formulas often can give a sufficient estimate of the interesting variables (Table 2). In this section estimates will be derived for the time-dependent return of magnetization toward steady state after a perturbation. These estimates will assume off-resonance conditions: $\omega \geq 314$ rad/s (50 Hz) and ${}^{E}R_2 \ll 314\ s^{-1}$ so that $\omega^2 + {}^{E}R_2^2 \approx \omega^2$, $c > 0$, and $c^{1/2} \gg b$. These

conditions are easily satisfied in most saturation-transfer experiments. It is also assumed that $f_0 = g_0 = 0$.

In some cases it will be convenient to present formulas in terms of the effective magnetic field in the rotating frame, $\mathbf{B}_e = (\omega/\gamma)\mathbf{k} + \mathbf{B}_1$ ($\mathbf{k}$ is a unit vector along the z axis). The angle θ between $\mathbf{B}_e$ and $\mathbf{B}_0$ is determined by

$$\sin\theta = \omega_1/\omega_e \quad [39]$$

$$\cos\theta = \omega/\omega_e, \quad [40]$$

where the effective precession frequency is

$$\omega_e = \gamma B_e = (\omega^2 + \omega_1^2)^{1/2}. \quad [41]$$

With the assumptions stated above, A and B in Eqs. [18] and [19] can be approximated by their Taylor series expansions:

$$A = c^{1/6}[1 - b/6c^{1/2} - b^2/36c + \text{smaller terms}] \quad [42]$$

$$B = -c^{1/6}[1 + b/6c^{1/2} - b^2/36c + \text{smaller terms}]. \quad [43]$$

The first and third terms of $A + B$ and the second term of $A - B$ cancel, leaving

$$A + B = -b/(3c^{1/3}) + \text{smaller terms} \quad [44]$$

$$A - B = 2c^{1/6} + \text{smaller terms} \quad [45]$$

$$AB = -a/3 = -c^{1/3} + \text{smaller terms}. \quad [46]$$

With $\omega \gg {}^E R_2$, Eqs. [21], [22], and [44]–[46] simplify to

$$b = ({}^{\sigma E}R_1 - {}^E R_2)[(2\omega^2 - \omega_1^2)/3 + \text{smaller terms}] \quad [47]$$

$$c = (\omega^2 + \omega_1^2)^3/27 + \text{smaller terms} \quad [48]$$

$$A + B \approx ({}^E R_2 - {}^{\sigma E}R_1)(2\omega^2 - \omega_1^2)/3(\omega^2 + \omega_1^2) \quad [49]$$

$$A - B \approx 2\omega_e 3^{1/2}/3 \quad [50]$$

$$AB \approx -\omega_e^2/3. \quad [51]$$

The differences between these estimates and the actual values with certain values of ω, ω_1, and R_2 are shown in Table 2. If ${}^{\sigma E}R_1 = {}^E R_2$, then $b = 0$ and Eqs. [48]–[51] are exact.

Substitution of Eq. [49] into Eq. [15] and rearranging yields an estimate of the effective spin–lattice relaxation rate constant $R_{1\text{eff}} = -\alpha_1$:

$$\alpha_1 \approx -[{}^{\sigma E}R_1 + ({}^E R_2 - {}^{\sigma E}R_1)\sin^2\theta]$$
$$= -(R_1 + k_{DE} + \sigma_{DE} + R_{1\text{RF}}) \quad [52]$$

$$\alpha_1 \approx -[{}^{\sigma E}R_1\cos^2\theta + {}^E R_2\sin^2\theta]. \quad [53]$$

This $R_{1\text{eff}}$ is a weighted average of ${}^{\sigma E}R_1$ and ${}^E R_2$, with weighting factors derived from the longitudinal (for ${}^{\sigma E}R_1$) and transverse (for ${}^E R_2$) components of $\mathbf{B}_e$, which is nearly parallel to the steady-state magnetization vector (see below). The contributions of R_1, chemical exchange, cross-relaxation, and irradiation to $R_{1\text{eff}}$ are approximately separable. Remember that these formulas assume that E_z is constant. A change in E_z while spin D relaxes will cause nonexponential relaxation (*37*).

Substitution from Eq. [49] into Eq. [16] and rearrangement gives the real part of α_2, which determines how fast the oscillations decay:

$$\text{Re}(\alpha_2) = -{}^E R_2[\cos^2\theta + 0.5(1 + {}^{\sigma E}R_1/{}^E R_2)\sin^2\theta] \quad [54]$$

$${}^E R_2/2 < -\text{Re}(\alpha_2) < {}^E R_2. \quad [55]$$

Thus the oscillations decay with a rate constant slightly less than ${}^E R_2$. The limit $-\text{Re}({}^E\alpha_2) = {}^E R_2$ is approached if ${}^{\sigma E}R_1 = {}^E R_2$ or if $\omega_1 \ll \omega$ ($\theta \approx 0$). The limit $-\text{Re}(\alpha_2) = {}^E R_2/2$ is approached if $\omega_1 \gg \omega$ ($\theta \approx \pi/2$) and ${}^E R_2 \gg {}^{\sigma E}R_1$.

Substituting from Eq. [50] into Eq. [16] shows that the imaginary part of α_2, which determines the oscillation frequency, is approximately ω_e:

$$\text{Im}(\alpha_2) \approx \omega_e. \quad [56]$$

The magnitude of the oscillations is determined by m_2, f_2, and g_2. The denominator of m_2, f_2, and g_2 is $d_2 = d_3^* = \alpha_2(\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)$. If $\omega \gg {}^E R_2$, then the real parts of α_2 and $\alpha_2 - \alpha_1$ are small and may be neglected (Eqs. [53]–[56]). After substitution from Eqs. [16], [17], [50], and [56],

$$d_2 = d_3^* \approx -2i(\omega^2 + \omega_1^2)^{3/2} = -2i\omega_e^3. \quad [57]$$

In the numerator of m_2 the real part of ${}^E R_2 + \alpha_2$ is small and may be neglected, and from Eq. [56] $[i\text{Im}(\alpha_2)]^2 \approx -(\omega^2 + \omega_1^2)$, so

$$\text{Num}(m_2) \approx -\omega_1^2({}^{\sigma E}M_{0D}{}^E R_1 + m_0\alpha_2). \quad [58]$$

If $m_0 = 0$,

$$m_2 \approx -i\,{}^{\sigma E}M_{0D}\,{}^{\sigma E}R_1\omega_1^2/2\omega_e^3 \ (m_0 = 0), \quad [59]$$

so $2|m_2|$ is very small. In addition $\phi \approx -\pi/2$, so that the cosine curve becomes a sine curve. Thus, the oscillations are small if $m_0 \approx 0$. If $m_0 \approx \pm M_{0D}$ the first term in Eq. [58] is small and may be neglected and from Eqs. [54]–[56] $\alpha_2 \approx i\ \text{Im}(\alpha_2)$. After substituting from Eq. [56],

$$m_2 \approx m_3 \approx m_0(\sin^2\theta)/2 \ (m_0 \approx \pm M_{0D}). \quad [60]$$

The oscillation phase is $\phi \approx 0$ if $m_0 = M_{0D}$ and $\phi \approx \pi$ if $m_0 = -M_{0D}$.

The magnitude of the exponentially decaying magnetization is determined by m_1, f_1, and g_1. The denominator in m_1, f_1, and g_1 is

$$d_1 = \alpha_1(\alpha_1 - \alpha_2)(\alpha_1 - \alpha_3) = 3\alpha_1[(A + B)^2 - AB]. \quad [61]$$

When $\omega \gg {}^{E}R_2$, comparison of Eq. [51] with Eq. [49] shows that $-AB \gg (A + B)^2$, so $d_1 \approx \alpha_1\omega_e^2$. After substituting from Eq. [53],

$$d_1 \approx -[{}^{\sigma E}R_1\omega^2 + {}^{E}R_2\omega_1^2]. \quad [62]$$

This is the negative of the denominator in the steady-state magnetizations with $\omega \gg {}^{E}R_2$, Eqs. [26], [30], and [34]. When spin–lattice relaxation measurements are made, the starting point is usually $f_0 = g_0 = 0$ and $m_0 = 0$ or $\pm M_{0D}$. With these assumptions and neglecting the M_{xE} and M_{yE} terms (which are small, see below) in the numerator of ${}^{i\sigma E}M_{0D}$ (Eq. [26]), Eq. [27] simplifies to

$$m_1 \approx -{}^{i\sigma E}M_{0D} + m_0\cos^2\theta. \quad [63]$$

With purely exponential relaxation, the magnitude of the exponential component is the difference between the initial magnetization m_0 and the steady-state magnetization ${}^{i\sigma E}M_{0D}$, or $m_0 - {}^{i\sigma E}M_{0D}$. With oscillations, the magnitude of the exponential component is decreased by the magnitude of the oscillation (see Eqs. [38] and [60]). The approximations are consistent with the requirement that the sum of the magnetization components at $t = 0$ equals m_0:

$${}^{i\sigma E}M_{0D} + m_1 + m_2 + m_3 = m_0. \quad [64]$$

Sample Spin System

Some of the formulas in the next sections will be demonstrated with a sample spin system, the frequently studied system of phosphocreatine (PCr), the gamma phosphate of adenosine triphosphate (ATP), and inorganic phosphate (*1–5, 8–10, 16*). We will examine the experiment where the ATP resonance is saturated (spin E) and the PCr signal is observed (spin D). The simulated data ignore spin–spin coupling, chemical shift ranges caused by imperfect shimming, and exchange of ATP with inorganic phosphate.

The calculated data assume typical T_1 and T_2 times for PCr and ATP (*41*): T_1(PCr) = 4 s, T_1(ATP) = 1.25 s, T_2(PCr) = 0.2 s, T_2(ATP) = 0.05 s. The T_1 values are not corrected for chemical exchange, which can cause significant errors (*23*), but they provide reasonable values to compare the different formulas for calculating k_{DE}. In addition M_0(PCr)/M_0(ATP) = 2, $k_{DE} = 0.5\ \mathrm{s}^{-1}$, and the frequency difference between PCr and ATP is 400 rad/s (~64 Hz or 2.5 PPM at 1.5 T).

OFF-RESONANCE EFFECTS ALONE

The solutions of the Bloch equations without chemical exchange or cross-relaxation can be found by setting $k_{DE} = k_{ED} = \sigma_{DE} = \sigma_{ED} = 0$. In this case ${}^{\sigma E}R_1 = R_1$ and ${}^{E}R_2 = R_2$.

Steady-State Magnetization

In the presence of a radiofrequency irradiation without chemical exchange, steady-state longitudinal magnetization is found from Eq. [26]:

$$\frac{{}^{i}M_{0D}}{M_{0D}} = \frac{R_1(\omega^2 + R_2^2)}{R_1(\omega^2 + R_2^2) + R_2\omega_1^2}, \quad [65]$$

a well-known result (*24, 33, 35, 36*). The ratio of the decrease in magnetization to the remaining magnetization is

$$\frac{M_{0D} - {}^{i}M_{0D}}{{}^{i}M_{0D}} = \frac{R_2\omega_1^2}{R_1(\omega^2 + R_2^2)}. \quad [66]$$

On resonance $\omega = 0$, and Eqs. [65] and [66] simplify to

$$\frac{{}^{i}M_{0D}}{M_{0D}} = \frac{R_1R_2}{R_1R_2 + \omega_1^2}\ (\omega = 0) \quad [67]$$

$$\frac{M_{0D} - {}^{i}M_{0D}}{{}^{i}M_{0D}} = \frac{\omega_1^2}{R_1R_2}\ (\omega = 0). \quad [68]$$

For the off-resonance spin in a saturation transfer measurement $\omega \gg R_2$, and Eqs. [65] and [66] simplify to

$$\frac{{}^{i}M_{0D}}{M_{0D}} \approx \frac{R_1\omega^2}{R_1\omega^2 + R_2\omega_1^2}\ (\omega \gg R_2) \quad [69]$$

$$\frac{M_{0D} - {}^{i}M_{0D}}{{}^{i}M_{0D}} \approx \frac{R_2\omega_1^2}{R_1\omega^2}\ (\omega \gg R_2). \quad [70]$$

Equation [70] shows that if $\omega \gg R_2$, the decrease in intensity caused by off-resonance irradiation depends only on the ratios R_2/R_1 and ω_1/ω.

The relative amounts of on-resonance and off-resonance saturation can be found by comparing Eqs. [68] and [70]. For given values of R_1, ω_1, and ω, a broad line (short T_2, large R_2) is more difficult to saturate on resonance and has a greater off-resonance effect. Thus, it is easier to saturate narrow peaks (long T_2, small R_2) without off-resonance effects.

Steady-state transverse magnetization can be calculated from Eqs. [30] and [34] with $k_{ED} = \sigma_{ED} = 0$. On resonance, $\omega = 0$ so ${}^{i}M_{xD}$(ss) = 0,

$${}^{i}M_{yD}(\mathrm{ss})/M_{0D} < R_1/\omega_1\ (\omega = 0) \quad [71]$$

$${}^{i}M_{yD}(\mathrm{ss})/{}^{i}M_{0D} = \omega_1/R_2\ (\omega = 0). \quad [72]$$

Therefore there may be a small amount of transverse magnetization along the y axis. Far from resonance, $\omega \gg R_2$, so

$$ {}^{\mathrm{i}}M_{xD}(\mathrm{ss})/{}^{\mathrm{i}}M_{0D} < \omega_1/\omega \qquad (\omega \gg R_2) \qquad [73] $$

$$ {}^{\mathrm{i}}M_{yD}(\mathrm{ss})/{}^{\mathrm{i}}M_{0D} < R_2\omega_1/\omega^2 \quad (\omega \gg R_2), \qquad [74] $$

where ${}^{\mathrm{i}}M_{0D}$ is smaller than M_{0D}. There may be considerable transverse magnetization, this time mostly along the x axis. In both cases these are the x and y axes in the reference frame rotating at the RF frequency, not at the resonance frequency of the spin. As expected (*15*), the steady-state magnetization is approximately aligned with $\mathbf{B}_{\mathrm{e}}$, although the alignment is not perfect as sometimes claimed (*42*).

Recovery of Longitudinal Magnetization

In the presence of an off-resonance irradiation, Eq. [23] shows that recovery of longitudinal magnetization M_z toward a steady state does not proceed in a purely monoexponential manner. If c in Eq. [22] is negative, there is a triexponential decay of M_z (*33, 40*). In most saturation-transfer experiments the irradiation is at least $\omega = 50$ Hz (314 rad/s) off resonance (*14, 42*), and this is much greater than $R_2 - R_1$. Under these conditions M_z recovery has a significant monoexponential component modulated by a decaying oscillatory component (Eq. [38] and Fig. 2). The initial magnitude of this oscillatory component, $2|m_2|$, depends on ω, ω_1, and the initial longitudinal magnetization m_0 (Eqs. [59] and [60]). If $m_0 \approx 0$ the oscillations are small (Eq. [59] and Fig. 2), and the exponential decay rate ${}^{\mathrm{i}}R_1$ can be considered $R_{1\mathrm{eff}}$. The exponential decay will be considered after the oscillatory component.

Oscillatory component. The initial amplitude of the oscillatory component, $2|m_2|$, is very small if $m_0 \approx 0$ (Eq. [59] and Fig. 2), as in progressive saturation and saturation recovery measurements of $R_{1\mathrm{eff}}$. The oscillation magnitude is approximately $m_0\sin^2\theta$ if $m_0 \approx -M_{0D}$, as in inversion recovery measurements (Fig. 2), or if relaxation from equilibrium toward the new steady-state is followed after the RF field is turned on ($m_0 \approx M_{0D}$) (Eq. [60]). The effects of the oscillations on the $R_{1\mathrm{eff}}$ measurement will be reduced further if the decay rate $-\mathrm{Re}(\alpha_2) \gg R_1$, which requires $R_2 \gg R_1$ (Eq. [55]). The oscillation frequency $\mathrm{Im}(\alpha_2) \approx \omega_{\mathrm{e}}$ (Eq. [56]). The phase offset of the oscillations (Eq. [38]) is usually near zero when $m_0 \approx M_{0D}$ and near π when $m_0 \approx -M_{0D}$ (Eq. [60]).

Exponential decay. The exponential decay constant ${}^{\mathrm{i}}R_1 = -\alpha_1$ is independent of the initial magnetization and depends only on R_1, R_2, ω, and ω_1 (Eqs. [15] and [18]–[22]). Equation [52] with $k_{DE} = \sigma_{DE} = 0$ shows that ${}^{\mathrm{i}}R_1$ is approximately R_1 plus a term that depends on ω, ω_1, and the difference $R_2 - R_1$. Equations [52], [53], and [69] can be rearranged to show that

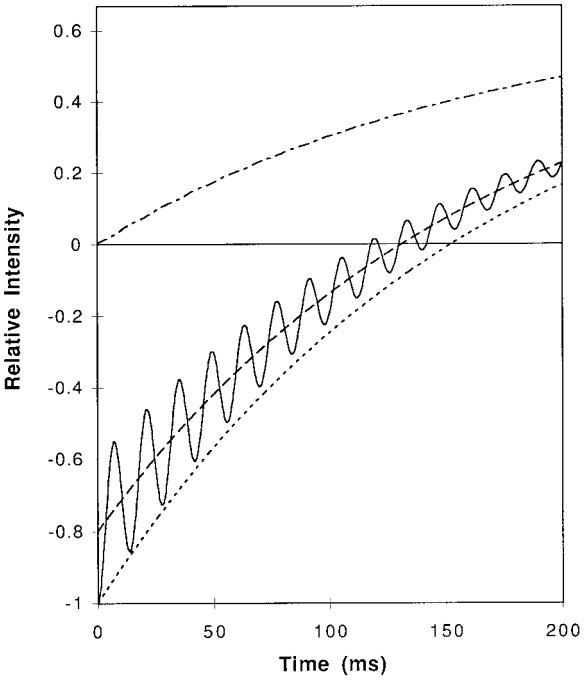

FIG. 2. Recovery of longitudinal magnetization during off-resonance irradiation. Dash–dotted line, recovery from $M_z = 0$. Solid line, recovery from $M_z = -1$. Dashed line, exponential component of recovery from $M_z = -1$. Dotted line, exponential recovery from $M_z = -1$ without off-resonance irradiation. The top of the graph is $0.6668 \approx {}^{\mathrm{i}}M_0$. The curves were calculated for $M_0 = 1$, $R_1 = 5\ \mathrm{s}^{-1}$, $R_2 = 10\ \mathrm{s}^{-1}$, $\omega = 400\ \mathrm{s}^{-1}$, and $\omega_1 = 200\ \mathrm{s}^{-1}$. These values were chosen to emphasize the oscillations.

$$ \frac{{}^{\mathrm{i}}M_{0D}}{M_{0D}} \approx \frac{{}^{\mathrm{i}}T_1}{T_1}\cos^2\theta \qquad [75] $$

$$ \frac{{}^{\mathrm{i}}R_1 - R_1}{{}^{\mathrm{i}}R_1} = \frac{T_1 - {}^{\mathrm{i}}T_1}{T_1} \approx \frac{(M_{0D} - {}^{\mathrm{i}}M_{0D})}{M_{0D}}\,\frac{(R_2 - R_1)}{R_2}. \qquad [76] $$

The fractional decrease in $T_{1\mathrm{eff}}$ is less than the fractional decrease in steady-state magnetization by a factor $(R_2 - R_1)/R_2$. If $R_1 = R_2$, ${}^{\mathrm{i}}R_1 = R_1$ so there is no change in $T_{1\mathrm{eff}}$. The fractional reduction in T_1 approaches the fractional reduction in M_{0D} only if $R_2 \gg R_1$.

The magnitude of the exponentially relaxing component is no longer $m_0 - {}^{\mathrm{i}}M_{0D}$, as it would be if there were no oscillations. The magnitude is decreased by approximately the magnitude of the oscillations (Eqs. [60] and [63] and Fig. 2). Furthermore, the intensity at $t = 0$ is offset from the exponential relaxation curve by approximately $m_0\cos^2\theta$. This can complicate T_1 measurements by inversion-recovery or by following the decrease in M_z after the RF field is turned on.

CROSS-RELAXATION

Without Off-Resonance Effects

With no chemical exchange or off-resonance effect, $k_{DE} = k_{ED} = 0$ and $\omega_1 = 0$. As long as M_{zE} is constant during the

measurement, the measured relaxation rate constant and steady-state M_{zD} are given by Eqs. [9] and [11], respectively:

$$R_{1\text{eff}} = {}^{\sigma}R_1 = R_1 + \sigma_{DE} \quad [77]$$

$${}^{\sigma}M_{0D} = M_{0D}(R_1 + \sigma_{DE}E_z)/(R_1 + \sigma_{DE}). \quad [78]$$

Application of Eq. [5] can convert this to the form commonly used for NOE measurements (*17, 18*):

$${}^{\sigma}M_{0D} = M_{0D} + (M_{zE} - M_{0E})\sigma_{ED}/(R_1 + \sigma_{DE}). \quad [79]$$

These formulas may be compared to those for a two-spin system, where there are equal numbers of D and E spins and $\sigma_{DE} = \sigma_{ED}$ (*17, 18*). The direct relaxation rate constant is $\rho = {}^{\sigma}R_1$ (Eq. [77]) and the cross-relaxation rate constant is $\sigma = -\sigma_{ED}$. The change in magnetization from M_{0D} to ${}^{\sigma}M_{0D}$, the NOE, may be positive or negative. A negative change probably accounts for a large part of the signal decrease in MRI with off-resonance irradiation, which partially saturates an invisibly broad proton population (*19–22*).

Incomplete saturation of spin E results in a proportional decrease in the signal intensity change of spin D, while ${}^{\sigma}R_1$ is not changed (as long as spin E magnetization is constant). This contrasts with the case of off-resonance effects alone, where the fractional decrease in $T_{1\text{eff}}$ was less than or equal to the fractional decrease in steady-state magnetization (Eqs. [75] and [76]).

With Off-Resonance Effects

Without chemical exchange, only the z component of magnetization exchanges by cross-relaxation between spins D and E. In this case the steady-state magnetization levels can be calculated from Eqs. [26], [30], and [34] or from

$${}^{i\sigma}M_{0D} = M_{0D}(\omega_D^2 + R_{2D}^2)K_{2D} \quad [80]$$

$${}^{i\sigma}M_{xD}(\text{ss}) = M_{0D}\omega_D\omega_1 K_{2D} \quad [81]$$

$${}^{i\sigma}M_{yD}(\text{ss}) = M_{0D}R_{2D}\omega_1 K_{2D}, \quad [82]$$

where

$$K_{2D} = \frac{R_{1D}[{}^{\sigma}R_{1E}(\omega_E^2 + R_{2E}^2) + R_{2E}\omega_1^2] + \sigma_{DE}R_{1E}(\omega_E^2 + R_{2E}^2)}{[{}^{\sigma}R_{1D}(\omega_D^2 + R_{2D}^2) + R_{2D}\omega_1^2] \times [{}^{\sigma}R_{1E}(\omega_E^2 + R_{2E}^2) + R_{2E}\omega_1^2] - \sigma_{DE}\sigma_{ED}(\omega_D^2 + R_{2D}^2)(\omega_E^2 + R_{2E}^2)}. \quad [83]$$

If $\sigma_{DE} = 0$, Eqs. [80]–[82] simplify to Eqs. [26], [30], and [34]. Equation [80] agrees with Eq. [2] of (*30*). The expression for K_{3D}, with three cross-relaxing spin populations, is given in Appendix A.

Equations [65]–[68] are valid with cross-relaxation if R_1, M_{0D}, and ${}^{i}M_{0D}$ are replaced by ${}^{\sigma}R_1$, ${}^{\sigma}M_{0D}$, and ${}^{i\sigma}M_{0D}$. In addition, after considering Eq. [78] and the modified Eq. [65],

$$\frac{{}^{i\sigma}M_{0D}}{M_{0D}} = \frac{(R_1 + \sigma_{DE}E_z)(\omega^2 + R_2^2)}{{}^{\sigma}R_1(\omega^2 + R_2^2) + R_2\omega_1^2}, \quad [84]$$

which reduces to the expression in Eq. [78] if $\omega_1 = 0$ and to Eq. [65] if $\sigma_{DE} = 0$.

CHEMICAL EXCHANGE

Cross-relaxation and chemical exchange have very similar effects both on spin–lattice relaxation rate constants (Eq. [9]) and on steady-state magnetization. The main difference is that chemical exchange transfers all three magnetization components while cross-relaxation transfers only M_z. Therefore there are k_{ED} terms without σ_{ED} terms in Eqs. [26]–[37], either directly or through Eqs. [13] and [14]. These k_{ED} terms arise from the k_{ED} terms in Eqs. [2] and [3]; the k_{DE} terms in those equations combine with R_2 to produce ${}^{E}R_2$ (Eq. [10]), and the k_{ED} term in Eq. [1] is included in ${}^{\sigma E}M_{0D}$ (Eq. [11]).

Without Off-Resonance Effects

In the simplest saturation-transfer experiment, the signal intensity of spin D is measured with $[{}^{E}M_{0D}(e)]$ and without saturation of spin $E(M_{0D})$, and ${}^{E}R_1$ is also measured. With no cross-relaxation or off-resonance effect, $\sigma_{ED} = 0$ and $\omega_1 = 0$. With constant M_{zE} the $R_{1\text{eff}}$ is ${}^{E}R_1 = R_1 + k_{DE}$ (Eq. [9]) and is independent of the exact value of M_{zE}. However, the steady-state longitudinal magnetization of spin D (Eq. [26]) decreases to ${}^{E}M_{0D}(e)$ (Eq. [11]),

$${}^{E}M_{0D}(e) = M_{0D}(R_1 + k_{DE}E_z)/(R_1 + k_{DE}). \quad [85]$$

Notice the similarity between Eqs. [78] and [85]. If spin E is fully saturated, $E_z = 0$ and Eq. [85] simplifies to the more familiar saturation-transfer equations (*3–7*), where

$${}^{E}M_{0D}(e)/M_{0D} = R_1/(R_1 + k_{DE}) = R_1/{}^{E}R_1 = {}^{E}T_1/T_1 \ (E_z = 0). \quad [86]$$

With complete saturation the fractional decrease in steady-state longitudinal magnetization equals the fractional decrease in $T_{1\text{eff}}$ since ${}^{E}M_{0D}(e)/M_{0D} = {}^{E}T_1/T_1$. This is equivalent to saying that the fractional decrease in steady-state magnetization is exactly balanced by the fractional increase in $R_{1\text{eff}}$ since $M_{0D}R_1 = {}^{E}M_{0D}(e){}^{E}R_1$. In this case the unidirectional rate constant k_{DE} can be calculated from

$$k_{DE} = {}^{E}R_1 - R_1 = {}^{E}R_1[M_{0D} - {}^{E}M_{0D}(e)]/M_{0D}. \quad [87]$$

TABLE 3
Magnetization Components under Different Exchange Conditions

	No exchange	Chemical exchange	Cross-relaxation
Spin E irradiated			
${}^{iE}D_x(e)$	0.0833	0.0320	0.0321
${}^{iE}D_y(e)$	0.0010	0.0004	0.0004
${}^{iE}D_z(e)$	0.8334	0.3195	0.3211
${}^{iD}E_x(e)$	0	0.0015	0
${}^{iD}E_y(e)$	0.0198	0.0273	0.0274
${}^{iD}E_z(e)$	0.0099	0.0143	0.0137
Control irradiation			
${}^{iE}D_x(e')$	−0.0833	−0.0877	−0.0880
${}^{iE}D_y(e')$	0.0010	0.0011	0.0011
${}^{iE}D_z(e')$	0.8334	0.8772	0.8801
${}^{iD}E_x(e')$	−0.0470	−0.0454	−0.0454
${}^{iD}E_y(e')$	0.0012	0.0011	0.0011
${}^{iD}E_z(e')$	0.9412	0.9078	0.9082

Note. Magnetization components were calculated for the creatine kinase spin system described in the text, with $\omega_1 = 40\ \mathrm{s}^{-1}$ and either $k_{DE} = 0$ (no exchange) or $k_{DE} = 0.5\ \mathrm{s}^{-1}$ (chemical exchange). The cross-relaxation values are those that would result if magnetization were transferred by cross-relaxation ($\sigma_{DE} = 0.5\ \mathrm{s}^{-1}$) instead of chemical exchange.

Alternatively, R_1 in the absence of exchange can be estimated (*23*), and k_{DE} can be calculated from (*1*),

$$k_{DE} = R_1[M_{0D} - {}^{E}M_{0D}(e)]/{}^{E}M_{0D}(e). \qquad [88]$$

If spin E is only partially saturated, $T_{1\mathrm{eff}}$ still equals ${}^{E}T_1$ as long as E_z is constant during the T_1 measurement, but the fractional decrease in steady-state magnetization is less than the fractional decrease in $T_{1\mathrm{eff}}$. In this respect chemical exchange is similar to cross-relaxation.

With Off-Resonance Effects

Effective spin–lattice relaxation rate. The effects of chemical exchange and off-resonance irradiation on $R_{1\mathrm{eff}} = -\alpha_1$ are approximately separable and additive (Eq. [52]). Notice that α_1 is independent of M_{zE}, so $R_{1\mathrm{eff}}$ is the same whether or not spin E is directly irradiated. However, this assumes that M_{zE} is constant during the measurement of $R_{1\mathrm{eff}}$, and this is nearly true when spin E is saturated but is not likely to be true when spin E is not irradiated directly. Also, if the relaxation begins with $m_0 \approx \pm M_{0D}$ rather than $m_0 \approx 0$, the oscillations may interfere with accurate measurement of α_1 (Eqs. [38], [59], and [60] and Fig. 2).

Steady-state magnetization with spin E irradiated. The steady-state magnetization formulas shown in Eqs. [26], [30], and [34] depend on M_{xE} and M_{yE}, whose values are not easily measured. However, Eqs. [71]–[74] seem to be good approximations even with chemical exchange (Table 3), so M_{xE} and M_{yE} can be expressed approximately in terms of M_{zE}.

There are several ways to estimate the steady-state magnetizations for specific values of the relaxation and exchange rate constants and relative equilibrium magnetizations. One solution is to set $dM/dt = 0$ in Eqs. [6]–[8] and in the three corresponding equations for spin E. The solution for these six equations with six unknowns can be determined numerically by standard mathematical computer programs and yields steady-state magnetizations for E as well as D. This exact answer is useful for calculating simulated data when the equilibrium intensities and relaxation rate constants for both spins are known. Analytic steady-state formulas can be calculated by this method, but they are much more cumbersome than the formulas with cross-relaxation (Eqs. [80]–[83] and [A6]–[A8]) and offer no helpful insights (*43*).

A second solution is to use equations analogous to Eqs. [26], [30], and [34] to calculate the steady-state values of M_{xE}, M_{yE}, and M_{zE} in the absence of exchange. These estimates can be inserted into Eqs. [26], [30], and [34]. This process can be repeated if desired, with the estimated M_{xD}, M_{yD}, and M_{zD} used for calculating new estimates of M_{xE}, M_{yE}, and M_{zE}, and so on.

A third solution is to use the formulas for cross-relaxation, ignoring the exchange of transverse magnetization (Eqs. [80]–[83] and [A6]–[A8]). This gives a reasonably good approximation in the creatine kinase sample spin system (Table 3) and can be used as a starting point for the iterative method just described. Notice that ${}^{iE}D_x(e)$ decreases in parallel with ${}^{iE}D_z(e)$ (Table 3), so that the steady-state magnetization remains nearly parallel to the effective field.

For deriving approximate analytic formulas, it is possible to recognize that M_{xE} and M_{yE} are small on resonance in the absence of chemical exchange (Eqs. [71] and [72] and Table 3) and set $M_{xE} = M_{yE} = 0$. The resulting formulas are similar to cross-relaxation formulas, except that R_2 is replaced by ${}^{E}R_2$. Transverse magnetization of a saturated spin can be very different with chemical exchange than without chemical exchange (Table 3), so the error in ${}^{iE}M_{0D}(e)$ can reach a few percent.

Steady-state magnetization with spin E not irradiated. When the control position is irradiated (Fig. 1C), the intensities of spins D and E may both decrease. Each spin may have significant $M_x(\mathrm{ss})$ (Eq. [73] and Table 3), while $M_y(\mathrm{ss})$ will be small (Eq. [74] and Table 3). The steady-state magnetizations may be calculated numerically or by iterative application of Eqs. [26], [30], and [34] to spins D and E. The error caused by setting $M_{xE} = M_{yE} = 0$ in Eqs. [26], [30], and [34] can reach a few percent and can be estimated by ignoring the M_{yE} term, which is much less than the M_{xE} term. After applying Eq. [73] to spin E with $\omega_E = 2\omega$, inserting this result into Eq. [26], and using Eqs. [4] and [11], the approximate fractional error in ${}^{iE}M_{0D}(e)$ from ignoring the M_{xE} and M_{yE} terms in Eq. [26] is

$$\text{fractional error} \approx (\omega_1^2 k_{DE} E_z)/[2\omega^2(R_1 + k_{DE}E_z)]. \qquad [89]$$

This error can reach a few percent and is limited by the fact that

as ω_1/ω increases, E_z decreases (Eq. [69]). Setting $M_{xE} = M_{zE}\omega_1/\omega_E$ in Eqs. [26], [30], and [34] will reduce the error (Eq. [73] and Table 3).

In saturation-transfer measurements of chemical exchange rate constants, the difference in M_{xE} and M_{yE} when spin E is irradiated compared to "control" irradiation means that the control is imperfect. Although the direct RF effects may be the same, the presence of M_{xE} and M_{yE} in Eq. [26] adds a new factor to ${}^{iE}M_{0D}$ during the control irradiation. This complication does not occur when magnetization is exchanged by cross-relaxation.

Baguet and Roby have suggested other formulas for estimating the steady-state M_z in the presence of RF irradiation and either chemical exchange or cross-relaxation (*13*). Their formulas assume either control irradiation or complete spin E saturation and are equivalent to

$$\frac{{}^{i\sigma}M_{0D}}{M_{0D}} = \cos^2\theta_D \frac{R_{1D}\,{}^{i\sigma}R_{1E} - R_{1E}\sigma_{DE}\cos^2\theta_E}{{}^{i\sigma}R_{1D}\,{}^{i\sigma}R_{1E} - \sigma_{DE}\sigma_{ED}\cos^2\theta_D\cos^2\theta_E} \quad [90]$$

$$\frac{{}^{iE}M_{0D}}{M_{0D}} = \cos\,\theta_D \frac{R_{1D}\,{}^{iD}R_{1E}\cos\,\theta_D - R_{1E}k_{DE}\cos\,\theta_E\cos(\theta_D - \theta_E)}{{}^{iE}R_{1D}\,{}^{iD}R_{1E} - k_{DE}k_{ED}\cos^2(\theta_D - \theta_E)}. \quad [91]$$

These equations simplify to Eq. [69] when there is no magnetization exchange and to Eqs. [78] and [86] when $M_{zE} = 0$ with no off-resonance effects. Values calculated with these formulas agreed very well with our calculations when spin E was nearly completely saturated. The increasing discrepancies as ω_1 decreased can be attributed to incomplete spin E saturation.

CALCULATING EXCHANGE RATE CONSTANTS

The preceding sections provide formulas to calculate both steady-state magnetization and $R_{1\text{eff}}$ in the presence of incomplete saturation and off-resonance effects. The ability of published formulas to estimate accurately the exchange rate constant k_{DE} can be calculated from simulated data generated with these equations. The following comparisons assume that cross-relaxation is absent ($\sigma_{DE} = 0$) and that the intensities and relaxation rates can be measured with no errors.

Published Saturation Transfer Formulas

Several formulas have been published for calculating k_{DE} from saturation transfer data (*1, 3, 4, 7, 9, 13, 14*). The formulas differ in their attempts to correct for off-resonance effects and incomplete saturation. Most formulas use ${}^{iE}R_1$, but it is also possible to use the intrinsic R_1 (*1*). The formulas will be repeated here in the current notation.

Forsén and Hoffman assumed that saturation was complete with no off-resonance effects, as in Eqs. [87] and [88] (*7*). Their formula requires measurement of M_{0D}, ${}^{iE}M_{0D}(e)$, and ${}^{iE}R_1$ (Eq. [92]). Equation [93] assumes $R_1/{}^{iE}R_1 = {}^{iE}M_{0D}(e)/M_{0D} = {}^{iE}D_z(e)$ (see Eqs. [11] and [86]):

$$\begin{aligned} k_{DE} &= {}^{iE}R_1[M_{0D} - {}^{iE}M_{0D}(e)]/M_{0D} \\ &= {}^{iE}R_1[1 - {}^{iE}D_z(e)] \end{aligned} \quad [92]$$

$$\begin{aligned} k_{DE} &= R_1[M_{0D} - {}^{iE}M_{0D}(e)]/{}^{iE}M_{0D}(e) \\ &= R_1[1 - {}^{iE}D_z(e)]/{}^{iE}D_z(e). \end{aligned} \quad [93]$$

The most common correction for off-resonance effects uses a control experiment, where the irradiation is placed on the opposite side of spin D from spin E an equal distance away (Fig. 1). The steady-state level of spin D, ${}^{iE}M_{0D}(e')$, is measured and is used as the equilibrium magnetization in Eqs. [92] and [93], yielding Eq. [94] (*3, 4*) or Eq. [95] (*1*):

$$k_{DE} = {}^{iE}R_1[{}^{iE}M_{0D}(e') - {}^{iE}M_{0D}(e)]/{}^{iE}M_{0D}(e') \quad [94]$$

$$k_{DE} = R_1[{}^{iE}M_{0D}(e') - {}^{iE}M_{0D}(e)]/{}^{iE}M_{0D}(e). \quad [95]$$

Potts *et al.* tried to correct for off-resonance effects by measuring both ${}^{i}R_1$ and ${}^{i}M_{0D}$ in the absence of exchange (*14*). This was possible in their system where the two sites were intracellular and extracellular, but usually such measurements are not possible. Their formula is equivalent to

$$k_{DE} = {}^{i}R_1[{}^{i}M_{0D} - {}^{iE}M_{0D}(e)]/{}^{iE}M_{0D}(e). \quad [96]$$

Baguet and Roby have proposed a complex procedure to correct for off-resonance effects (*13*). This method, which involves measuring $R_{1\text{eff}}$ and $R_{2\text{eff}}$ for both spins and requires knowledge of ω_1, is not easily expressed in a single equation. A simpler attempt to correct for both off-resonance effects and incomplete saturation (*9*) uses the formula

$$\begin{aligned} k_{DE} = R_1[{}^{iE}D_z(e') - {}^{iE}D_z(e)]/ \\ [{}^{iE}D_z(e) - {}^{iE}D_z(e') + {}^{iD}E_z(e') - {}^{iD}E_z(e)]. \end{aligned} \quad [97]$$

Example

The accuracy of published formulas was compared in the creatine kinase spin system described above. Three RF strengths were examined: $\omega_1 = 15$ rad/s for incomplete saturation, $\omega_1 = 40$ rad/s for significant off-resonance effects, and $\omega_1 = 120$ rad/s for large off-resonance effects. The resulting intensities and relaxation rate constants are shown in Table 4. The percentages of error in k_{DE} calculated by the different methods are shown in Table 5. These calculations assume noiseless data, so that even very small values of residual spin E magnetization can be measured accurately.

Equations [92] and [93], with no correction for off-resonance effects or incomplete saturation, have significant errors in the calculated k_{DE}. The common correction for off-reso-

TABLE 4
Simulated Data for Measuring k_{DE}

Calculated value	ω_1 (s^{-1}) 15	40	120
Saturation of spin E + control			
${}^{iE}D_z(e')$	0.9808	0.8772	0.4320
${}^{iD}E_z(e')$	0.9857	0.9078	0.5507
${}^{iE}D_z(e)$	0.3928	0.3195	0.2017
${}^{iD}E_z(e)$	0.0953	0.0143	0.0015
${}^{i}D_z$	0.9726	0.8334	0.3572
${}^{iE}R_{1D}$ (s^{-1})	0.7567	0.7970	1.1422
${}^{i}R_{1D}$ (s^{-1})	0.2567	0.2970	0.6422
Saturation of spin D + control			
${}^{iE}D_z(d')$	0.9802	0.8783	0.5009
${}^{iD}E_z(d')$	0.9735	0.8374	0.3591
${}^{iE}D_z(d)$	0.0113	0.0015	0.0001
${}^{iD}E_z(d)$	0.4435	0.3990	0.2176

nance effects is reasonably good with Eq. [94], but the answer with Eq. [95] may be less accurate than with no correction. Neither of these methods corrects for incomplete saturation. Equation [97] compensates reasonably well for incomplete saturation, but not for off-resonance effects. The complex method of Baguet and Roby (*13*) partially compensates for off-resonance effects, but not for incomplete saturation. Equation [96], which is intended to correct for off-resonance effects on both signal intensity and R_1, compensates well for the off-resonance effect but not for incomplete saturation.

DISCUSSION

Solutions of Modified Bloch Equations

The steady-state solution of the Bloch equations without chemical exchange or cross-relaxation was included in the original paper (*24*), and an interesting geometrical steady-state solution also has been published (*44*). Torrey derived time-dependent changes with constant RF power and frequency in certain special cases ($\omega = 0$, $R_1 = R_2$, or $R_2 - R_1 \ll \omega_1$) (*25*), and some mistakes in that paper have been corrected (*34*). Hore and McLauchlan provided a general analytic solution for the equations assuming no initial transverse magnetization (*40*). The complete analytic solution derived by extending the Laplace transform method (*33*) is the basis for the present work. Solutions also have been derived by other methods (*35, 36, 45*).

With chemical exchange (*32*) Forsén and Hoffman derived steady-state longitudinal magnetization and $R_{1\text{eff}}$ when one exchanging spin was completely saturated with no off-resonance effects (neglecting transverse magnetization) (*7*), and this was later extended to three exchanging spins (*10–12*). Schotland and Leigh solved the modified Bloch equations with exchange among n sites when $\omega_1 = 0$ (*37*).

Cross-relaxation (*18*) has been considered in MRI, where two populations of H_2O have the same or very similar resonance frequencies and very different linewidths because of different T_2 relaxation times (*19–22*). Solutions have been published for the steady state without off-resonance effects (*19*) and with off-resonance effects when $\omega_D = \omega_E$ (*20*) and for the transient magnetization without off-resonance effects (*21*). A general semi-analytic solution inserts the numerical solution to a sixth-order polynomial into analytic formulas (*22*). Caines and Schleich and co-workers considered off-resonance effects with M_z exchange in two cases, ignoring the transient oscillations and presenting an equation that appears to be equivalent to Eq. [75] in the present work (*29, 30*).

Chemical exchange and cross-relaxation between two sites have been considered together without RF irradiation (*46*). The formulas presented here provide both transient and steady-state formulas with chemical exchange, cross-relaxation, and direct off-resonance effects. The accuracy of the approximate formulas in Eqs. [49] to [63] and [69] was checked over a range $320 \leq \omega \leq 8640$ rad/s (~51–1375 Hz) and $20 \leq \omega_1 \leq 540$ rad/s (~3–86 Hz), and $1 \leq R_2 \leq 100$ s^{-1} (Table 2). This represents the usual range of frequency differences encountered in saturation-transfer measurements of chemical exchange. With a solid-like component in magnetization transfer, R_{2E} can be very large, and the approximate formulas would apply only to spin D, not spin E.

Comparison of Cross-Relaxation and Chemical Exchange

Exchange of magnetization between two populations of nuclear spins may occur by cross-relaxation or by chemical exchange. Although the physical transfer mechanisms are different, they have identical effects on z magnetization transfer (Eq. [1]) and on the effective spin–lattice relaxation rate constant (Eq. [52]). One difference between these two effects is that when spin E is saturated spin D intensity may increase or decrease with cross-relaxation, while it always decreases with

TABLE 5
Percent Errors in k_{DE} Calculated by Different Methods

Ref.	Eq.	ω_1 (s^{-1}) 15	40	120
7	92	−8	8	82
7	93	−23	6	98
3	94	−9	1	22
1	95	−25	−13	−43
14	96	−24	−4	−1
9	97	−3	−17	−64
13	—	−24	−5	−22

Note. The percentage of difference between the k_{DE} calculated by each method and the true $k_{DE} = 0.5$ s^{-1} is shown for three different values of ω_1 (rad/s) in the reaction catalyzed by creatine kinase.

chemical exchange. The convention used here, with a positive σ value leading to a decrease in intensity when spin E is saturated, is the opposite of standard NOE experiments. Another difference is that transverse magnetization is transferred in chemical exchange but not in cross-relaxation (Eqs. [2] and [3]). Thus transverse spin E magnetization directly affects spin D intensity with chemical exchange but not with cross-relaxation (Eq. [26]).

Steady-State Magnetization

At steady state with one RF field the magnetization vector is approximately aligned with the effective field, so that the small force applied by that field is balanced by R_1 and R_2 effects. With chemical exchange the transverse components can exchange between spins D and E, and this exchange appears in Eqs. [2], [3], [7], [8], [13], [14], and [26]–[37]. Chemical exchange and cross-relaxation produce similar values of M_x and M_y, but with chemical exchange there may be a small amount of M_x on resonance (Table 3).

The x and y components that appear in these equations refer to the x and y axes of the reference frame rotating at the RF frequency, not at the spin's resonant frequency. The steady-state x and y components of spin D and spin E in the RF reference frame are constant, so they must be rotating in the spin D and spin E reference frames. With a second RF field the steady-state M_z oscillates about an average value.

These results are valid for a constant-amplitude irradiation. If saturation is achieved by a pulsed irradiation (*4, 14*), the exact saturation method must be considered.

Effective Spin–Lattice Relaxation

We assume that the presence of an RF field does not change the intrinsic relaxation process, which remains monoexponential (*47*). However, the RF field interacts with both longitudinal and transverse magnetization, changing the rate ($R_{1\text{eff}}$) at which M_z approaches a steady state. The decrease in $T_{1\text{eff}}$ with off-resonance irradiation has been observed experimentally (*14, 28*). In the presence of RF irradiation, M_z recovery is not purely monoexponential. In most cases a large exponential component has additional oscillations which decay with time and vanish in the steady state (Fig. 2). The oscillations can be minimized by starting from no net magnetization rather than from fully relaxed or inverted magnetization (Fig. 2). Therefore if the large monoexponential component is to be considered the "true" effective T_1, its value is estimated better with progressive saturation (*3*) or saturation recovery methods than by inversion recovery (*2*) or by following the decrease in magnetization after the RF power is turned on (*8*). Although these oscillations have been predicted (*25, 33, 35, 36, 40*) and observed (*25, 33, 40*), the idea of oscillations interfering with T_1 measurements does not appear to have been mentioned (*48*).

For heterogeneous systems, such as perfused organs, there may be some advantage to measuring T_1 from the rate of intensity loss in spin D when spin E is irradiated. This eliminates the possible problem of two pools of a chemical, one exchanging and one not, with different T_1 times. By following the intensity loss when spin E is irradiated, only the exchanging spins contribute to the T_1 measurement. With progressive saturation both populations will contribute to the T_1 measurement. Although the formula for calculating actual exchange rates from the exchange rate constants corrects for the intensity of the nonexchanging pool, the T_1 measurement may not be completely valid. The relative advantages of each method must be considered in such a situation.

Measurement of $R_{1\text{eff}}$ requires that spin E magnetization be held constant during the R_1 measurement. This is nearly true when saturation is nearly complete, because the saturation occurs soon after the RF pulse is turned on. Moreover, the 90° detection pulse in a progressive saturation or saturation recovery experiment nulls M_{zE}, and during data acquisition M_{zE} may recover to a low level that approximates the saturated level. When saturation is incomplete, M_{zE} may change during the relaxation measurement, yielding an inaccurate estimate of $R_{1\text{eff}}$.

Relative Changes in Magnetization and Relaxation

The steady-state longitudinal magnetization $M_{zD}(\text{ss})$ can be affected by cross-relaxation, chemical exchange, and off-resonance effects. With chemical exchange and cross-relaxation, the measured $T_{1\text{eff}}$ is independent of M_{zE} as long as M_{zE} is constant during the measurement. However, $T_{1\text{eff}}$ differs from T_1 in the absence of chemical exchange and cross-relaxation. The fractional change in T_1 is equal to the fractional change in $M_{zD}(\text{ss})$ if spin E is completely saturated, otherwise the T_1 change is greater than the $M_{zD}(\text{ss})$ change (Eqs. [85] and [86]). With off-resonance effects alone, the T_1 change is less than the $M_{zD}(\text{ss})$ change (Eq. [76]).

Saturation-Transfer Measurements of Chemical Exchange

In some cases it may be possible to measure exchange rates without off-resonance effects, by using two-dimensional methods or lineshape analysis. With saturation-transfer methods off-resonance effects may occur. Previously published formulas for calculating k_{DE} did not give accurate results in all cases (Table 5). This is true even for formulas intended to correct for off-resonance effects (*1, 3, 9, 13, 14*) and incomplete saturation (*9*). In some cases the "corrected" k_{DE} values were less accurate than values calculated with the assumption of complete saturation and no off-resonance effects (Eq. [92]). This emphasizes the importance of accounting correctly for the effects of RF irradiation on both the steady-state magnetizations and the effective relaxation rates.

There is unlikely to be a single simple formula that will

provide an exact value of k_{DE} in all cases with off-resonance effects and incomplete saturation. The method of Potts *et al.* appears to give accurate results if saturation is complete, but this requires measurements in the absence of exchange (*14*). Such measurements usually are not possible without altering the relaxation or exchange rate constants. The equations derived here can provide a starting point for determining formulas that provide good estimates of k_{DE} in most cases of two-site exchange (*49*) and three-site exchange (P. B. Kingsley and W. G. Monahan, submitted for publication).

APPENDIX A

In many cases magnetization is exchanged between three spins, not just two. When spin D exchanges with spin E and a competing spin C, the equations must have an additional term added for each term containing an E (k_{DE}, k_{ED}, σ_{DE}, σ_{ED}, $M_{x,y,zE}$, $E_{x,y,z}$) with the E replaced by C. For example, k_{DE} becomes $k_{DE} + k_{DC}$, and $k_{ED}M_{xE}$ becomes $k_{ED}M_{xE} + k_{CD}M_{xC}$. Equations [9], [10], [11], [13], and [14] become

$$^{\sigma EC}R_1 = R_1 + k_{DE} + k_{DC} + \sigma_{DE} + \sigma_{DC} \quad \text{[A1]}$$

$$^{EC}R_2 = R_2 + k_{DE} + k_{DC} \quad \text{[A2]}$$

$$^{\sigma EC}M_{0D} = M_{0D}[R_1 + (k_{DE} + \sigma_{DE})E_z + (k_{DC} + \sigma_{DC})C_z]/^{\sigma EC}R_1 \quad \text{[A3]}$$

$$\alpha_i^{EC}f_0 = \alpha_i f_0 + k_{ED}M_{xE} + k_{CD}M_{xC} \quad \text{[A4]}$$

$$\alpha_i^{EC}g_0 = \alpha_i g_0 + k_{ED}M_{yE} + k_{CD}M_{yC}. \quad \text{[A5]}$$

These formulas are valid for a single RF frequency. With two RF frequencies, as in multiple saturation transfer (*3, 4, 10–12*), a single frequency must be chosen for the rotating reference frame, leading to a more complex analysis. In this case there is no true steady-state magnetization and all three components of magnetization oscillate in any reference frame.

When three spins exchange magnetization with each other by cross-relaxation, K_{2D} in Eq. [83] must be replaced by K_{3D}. To simplify notation let

$$[D] = {}^{\sigma}R_{1D}(\omega_D^2 + R_{2D}^2) + R_{2D}\omega_1^2 \quad \text{[A6]}$$

with the D subscripts changed to C for $[C]$ and to E for $[E]$ (see Eq. [83]). Then K_{3D} = Num/Den, where

$$\begin{aligned}\text{Num} = {} & R_{1D}\{[E][C] - \sigma_{CE}\sigma_{EC}(\omega_E^2 + R_{2E}^2)(\omega_C^2 + R_{2C}^2)\} \\ & + R_{1E}(\omega_E^2 + R_{2E}^2)\{\sigma_{DE}[C] + \sigma_{DC}\sigma_{CE}(\omega_C^2 + R_{2C}^2)\} \\ & + R_{1C}(\omega_C^2 + R_{2C}^2)\{\sigma_{DC}[E] + \sigma_{DE}\sigma_{EC}(\omega_E^2 + R_{2E}^2)\}\end{aligned} \quad \text{[A7]}$$

$$\begin{aligned}\text{Den} = {} & [D]\{[E][C] - \sigma_{CE}\sigma_{EC}(\omega_E^2 + R_{2E}^2)(\omega_C^2 + R_{2C}^2)\} \\ & - (\omega_D^2 + R_{2D}^2)\{\sigma_{DE}\sigma_{ED}(\omega_E^2 + R_{2E}^2)[C] \\ & + \sigma_{DC}\sigma_{CD}(\omega_C^2 + R_{2C}^2)[E] \\ & + (\sigma_{DE}\sigma_{EC}\sigma_{CD} + \sigma_{DC}\sigma_{CE}\sigma_{ED}) \\ & \times (\omega_E^2 + R_{2E}^2)(\omega_C^2 + R_{2C}^2)\}.\end{aligned} \quad \text{[A8]}$$

K_{3D} is symmetric with respect to spins C and E, reduces to K_{2D} (Eq. [83]) when spin C does not exchange ($\sigma_{DC} = \sigma_{EC} = \sigma_{CD} = \sigma_{CE} = 0$), and reduces to $R_{1D}/[D]$ if spin D does not exchange ($\sigma_{DE} = \sigma_{ED} = \sigma_{\mathrm{DC}} = \sigma_{\mathrm{CD}} = 0$). Formulas for K_{3C} and K_{3E} can be derived by cyclic permutation of the C, D, and E labels.

APPENDIX B

Errors in Eqs. [2c], [3], [37], [41], and [45] of Torrey (*25*) have been corrected (*34*; Eqs. [3], [4], [8], [20], and [40], respectively). A mistake in Eq. [39] of (*34*) was corrected in a published correction (the third term in the numerator is positive, not negative). In Eq. [58] of Torrey (*25*), the formula for A is really for D (see his Eq. [50c]), and $A = -\delta^2(1 - m_0)/(1 + \delta^2)$.

The correct version of Eq. [17c] in (*33*) can be found by setting $k_{DE} = \sigma_{DE} = 0$ in Eq. [22] of the present work:

$$c = \{(\omega^2 + \omega_1^2)^3 + (R_1 - R_2)^2[\omega^2(2\omega^2 + \{R_1 - R_2\}^2) - \omega_1^2(\omega_1^2/4 + 5\omega^2)]\}/27. \quad \text{[B1]}$$

In (*35*) γ^1 should be γ' in the line before Eq. [10], and the numerator of Eq. [17] should be $(1 + \delta^2T_2^2)M_0$ instead of $1 + \delta^2T_2^2M_0$. This equation is given correctly in Table 4 of their later paper (*36*). Table 8 of (*36*) should have $A_{33} = e^{-t/T_1}$ and $B_{33} = 1 - e^{-t/T_1}$.

In (*8*), the second $M_0(B)$ in Eq. [14] should be $M(B)$, and a corrected version of Fig. 1 was published.

In Eq. [3b] of (*19*), R_{AB} should be R_{BA}.

In Eqs. [1a, 1b] of (*21*), $-\beta_{A,B}\mathrm{M}_{A,B}^y$ should be $-\beta_{A,B}\mathrm{M}_{A,B}^x$.

In (*17*) in the middle of page 209, $\omega^2\tau_c \ll 1$ should be $\omega^2\tau_c^2 \ll 1$.

In the line after Eq. [26] of (*29*), $M_{ze}^A(1 + k_AT_{1\rho}^{\mathrm{off}}$ should be $M_{ze}^A(1 + k_A)T_{1\rho}^{\mathrm{off}}$.

In the Fig. 1 legend in (*13*), $\omega_{\mathrm{sym}} = 2\omega_{\mathrm{A}} - \omega_{\mathrm{B}}$, not $2\omega_{\mathrm{B}} - \omega_{\mathrm{A}}$. In Eq. [40] of (*13*), the T_{ZA} should be removed from the numerator.

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