to account for the $T_2$ observed. Thus, the proposed irrotationally bound water is unnecessary. Furthermore, if the irrotationally bound water indeed exists and is different from the nonfreezing bound water, the correct treatment of the problem should involve a calculation of the exchange between three fractions of water molecules. Equation 1 of Foster et al. is not correct.

4) If one argues that the irrotationally bound water is part of the nonfreezing water, and the $T_2$ relaxation of the nonfreezing water at $-34^\circ$C is dominated by roughly 1 percent irrotationally bound water so that the mobility of the other 99 percent is extremely great, there is still a serious discrepancy. Consider the relaxation time at $-34^\circ$C from equation 1 of Foster et al.

$$T_{2\text{obs}} = \frac{x}{P_h T_{2n}} + \tau_a$$

where $P_h$ is the fraction of irrotationally bound water, $\tau_a$ is the average lifetime of nonfreezing water with large mobility. $T_{2n}$ is the proton $T_2$ of irrotationally bound water, and $x = 1.0$ for proton water. Since the first term on the right side of equation 1 is larger than zero, $\tau_a < T_{2\text{obs}}$. At $-34^\circ$C $T_{2\text{obs}}$ is about 100 $\mu$s; so $\tau_a$ must be smaller than 100 $\mu$s at that temperature. But according to figure 3 in (1) the average lifetime of a water molecule not in the irrotationally bound phase is about 35 msec at room temperature. Since the nonfreezing water with large mobility is about 10 percent of the total tissue water, by detailed balance, $\tau_a$ must be approximately 3.5 msec. This means that $\tau_a$ increases with temperature. However, this result is opposite to the known temperature dependence of $\tau_a$ in liquid water and would imply a negative activation energy for water.

5) Finally, in figure 3 of Foster et al. the plotted data do not agree with their prediction. Regardless of the rate of exchange, their equation 1 should predict a straight line in the plot of $T_{2\text{obs}}$ against $x$. While the data seem to show a large degree of curvature. The curve probably results from a failure of equation 1 to include proton relaxation which is independent of the isotopic composition of the water, such as interaction between water protons and nonexchangeable macromolecular protons (4). In general, the $T_2$ of bound water can be calculated from

$$T_{2\text{bound}} = \frac{1}{T_{2\text{obs}}} = \frac{1}{a + f H} + \frac{1}{L} (1 - f H)$$

where $a$ is the relaxation effect from macromolecular protons, $\beta$ is the relaxation from intramolecular dipolar interaction, $L$ is the ratio of second moments between proton and deuteron, and $f_H$ is the isotopic fraction of protons in tissue water. Our $\beta$ here is equivalent to the inverse of $T_{2n}$ in equation 1 of (1). A comparison of the equation given above with that of Foster et al. indicates that their $x$ should be defined as

$$x = \frac{1}{a + b f_H}$$

where $a = \alpha/\beta + 1/L$ and $b = 1 - 1/L$. Foster et al. defined $x$ as $1/f_H$. If $a$ is significantly large in comparison with $b f_H$, it is not surprising that the graph of $T_{2\text{obs}}$ against $x$ deviates from a straight line.

In conclusion, we find that there are serious contradictions between the results of the analysis of Foster et al. and the experimental observations. Their basic assumption was unsound, and their conclusion as stated in their abstract was misleading. A casual reader may be misled to believe that only 0.1 percent of muscle water is bound. It was not clear in the report by Foster et al. how they differentiate between the observed nonfreezing bound water and the theoretical irrotationally bound water. We find that, in all cases, the approach adopted by Foster et al. would lead to results contradicting the observations on the nonfreezing water, the relaxation effect of which was neglected in their treatment.

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References and Notes
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The current situation with respect to the theory of nuclear magnetic resonance (NMR) phenomena in biological waters remains one of model building and hypothesis testing. Chang and Woessner (1) evidently believe that our recent research report on certain NMR phenomena of water in muscle (2) advocates too strongly a model with which they do not fully agree, mainly because of the narrow range of phenomena they see the model to encompass.

The model (2, 3) of intracellular water in question is that of relatively mobile water exchanging with a small number of bound water molecules (less than one per thousand); the bound molecules are held in a fixed orientation (irrotationally) for an average binding time, $\tau_n$, of several microseconds, such that $\tau_n$ is identically the NMR correlation time in the bound state; the binding time or lifetime in the bound state is sufficiently long that fast exchange [in the NMR sense (4)] between the two sorts of molecules does not occur. We refer to this as the model of irrotationally bound water (IBW) with binding-time effects. In the IBW model there are only two free parameters: the number of bound molecules and the lifetime in the bound state. The experiments we reported (2) were novel in that both parameters could be determined. For consistency with NMR theories of motional narrowing, the binding time is required to be (3) [and is found to be (2, 3, 5)] on the order of several microseconds. The IBW model also predicts the value of the applied radio-frequency field at which a dispersion of the rotating-frame spin lattice relaxation time $T_1$ occurs (3), as discussed further below. The significance of this model is that if it is correct, then NMR relaxation experiments tell very little about the mobility of most of the water molecules in muscle tissue, but reflect only the influence of a very few binding sites.

The two-fraction fast exchange (TFE) model, which has routinely been applied to water in biological systems (6, 7), may also have as few as two parameters, but one (say the bound fraction) must be chosen before the other (the mobility of the bound state) can be determined. In points 2, 3, and 4 of their comment, Chang and Woessner assume that the nonfreezing water, reported by others (6, 7) as well as in (2), is in some way the bound water fraction required by any TFE model and criticize the IBW model for not being in harmony with their interpretations. Certainly we studied the nonfreezing water, showed that it exchanges with $D_2O$, and thereby showed that it is not identical to the observed proton fraction with a $T_2$ in the millisecond range (2); thus bounds were placed on some commonly held notions of bound water. But this does not require us to devise a model which at once explains the NMR properties of unfrozen tissue and any
changes in these properties on freezing. We do not know how the NMR relaxation data in frozen tissue relate to NMR relaxation in unfrozen tissue; we do not know the mechanism of freezing or the alterations in structure and segregation caused by freezing. Thus, we feel neither competent nor obliged to respond to points 2, 3, and 4 above.

In their fifth criticism, Chang and Woessner correctly point out (i) that our model neglected the effect of dipolar interaction of the protons in the irrotationally bound water molecules with the protons in the macromolecular structure (8), and (ii) that inclusion of this effect might bring the model into better agreement with the data in the isotope dilution experiment (2). This has also been pointed out to us by Edzes and Samulski (5), who included this intermolecular contribution to $T_2$ for water molecules in the bound state in fitting our proton $T_2$ data for barnacle muscle, extracted its value, and found that it almost dominates the dependence on deuterium concentration in a manner consistent with rapid exchange. They pointed out that it is only the closeness of the deuterium and proton $T_2$'s which requires the introduction of the effects of $\tau_b$ and allows determination of the free parameters in the IBW model. Inclusion of the intermolecular contribution does not disturb the validity of the "incipient motional narrowing" consistency check (3) referred to above. Edzes and Samulski reworked the barnacle $T_2$ data because they were able to derive, from cross-relaxation effects in the relaxation time $T_1$ (albeit of water in chicken muscle), an independent estimate of the intermolecular contribution to $T_2$ in the bound state; the latter agrees very well with that found from the isotope dilution measurements in barnacle muscle and suggests that the model they (and Chang and Woessner) propose for isotopic dilution is valid. The cross-relaxation effects also require (5) that water molecules be bound for times $\tau_b$ greater than a Larmor period (that is, $\tau_b \geq 10^{-8}$ second); the parameters obtained from barnacle muscle (2, 3, 5) are consistent with this requirement ($\tau_b \sim 10^{-5}$ second). Inclusion of intermolecular contributions to $T_2$ for the protons of water molecules in the bound state does not therefore vitiate the IBW model, but rather sustains it.

It was pointed out (1–3) that the IBW model does not hold for protein solutions (9) and agar gels (10). We believe that these two systems may be sufficiently different from muscle tissue that the same theoretical model should not be required to explain the NMR properties of all of them. The rigid substrate (rigid for, say, tens of microseconds) required for the IBW model may be present in muscle and not in agar gels or protein solutions; it is, in fact, the purpose of the NMR experiments to ascertain these things. With regard to the $T_1$ dispersion data of Held et al. (11), we find support rather than contradiction of the model in question. As far as $T_{1w}$ and $T_1$ dispersion effects are concerned, the muscle systems are, as far as the model, effectively in the exchange rate–limited relaxation regime $T_1$, where dispersion reflects a local field or "root interaction strength" rather than a correlation time. The dispersion observed by Held et al. occurs as predicted (3) and as observed for frog (13) and mouse (14) muscle; further, the recent observation by Fung (15) that the proton and deuterium dispersion frequencies are not equal (as a fast-exchange model with a single motional process would require) but differ by a factor of $\sim 3$ is in agreement with this idea, as is the absolute value of the deuteron dispersion frequency (16).

The IBW model of one water molecule per thousand, briefly and irrotationally bound, can indeed account for a great many of the NMR properties of water in muscle, namely (i) the small value of $T_2$ generally observed for intracellular water; (ii) the dependence of the proton $T_2$ on isotopic composition in barnacle muscle, as extended by Chang and Woessner (1) and by Edzes and Samulski (5); (iii) the ratio of the deuterium transverse relaxation time to that of the protons in the same system; (iv) the dispersion of the rotating-frame relaxation times in mouse and frog muscle; and (v) the dispersion of both proton and deuteron spin lattice relaxation times in frog and mouse muscle. In addition, $T_1$ cross-relaxation effects in the intracellular water of chicken muscle (5) are consistent with the model as well (17).

In conclusion, we believe that the nonfreezing water which is often described as bound water (6, 7) is not immediately relevant to the construction of an NMR model for nonfrozen tissue; the model of a small fraction of water, irrotationally bound and exchanging at a fast to intermediate rate ($\sim 10^8$ sec$^{-1}$), is consistent with a rather large number of phenomena observed in various muscle systems; there are no serious contradictions between the results of the analysis given (2, 3) (as amended to include intermolecular effects) and the experimental observations originally presented.

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8. We neglected this effect in order to introduce the variable $x$, which made it possible to display the deuteron relaxation time in the same graph with the proton relaxation times (as a function of isotopic composition).
16. It appears that the $T_{1w}$ and $T_1$ dispersions do not have the shape characteristic of a single correlation time and that a distribution may be required; such a distribution may also account for the weak temperature dependence of $T_1$ (3, 12) and $T_2$ (5). See H. A. Resing, Adv. Mol. Relaxation Processes 1, 109 (1967–1968).
17. It is assumed in the paper that all muscle systems are approximately equivalent in terms of NMR relaxation properties and that the different experiments done on different systems can be combined as representing a single system.

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Rate-Dependency Hypothesis

In their recent article "Mathematics underlying the rate-dependency hypothesis," Gonzalez and Byrd (1) make precise and explicit some facets of rate dependency that had been merely implicitly understood. They are concerned with ways of presenting behavioral results when the independent variable is rate of responding and rates have been recorded in the presence and absence of a drug.

1) By using the word hypothesis they emphasize that the relation between rate
"Bound Water" in Barnacle Muscle as Indicated in Nuclear Magnetic Resonance Studies

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