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 τ_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 τ_b greater than a Larmor period (that is, $\tau_b \gtrsim 10^{-8}$ 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 $T_{1\rho}$ and T_1 dispersion effects are concerned, the muscle systems are, according to the model, effectively in the exchange rate–limited relaxation regime (*3*, *12*) 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 deuteron dispersion frequencies are not equal (as a fast-exchange model with a single motional process would require) but differ by a factor of ~ 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 ($\sim 10^5$ 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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2 September 1977

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

of responding and drug effect has come to be regarded as more than a description of a familiar finding; it is now widely hypothesized that the rate, to a greater or lesser extent, determines the drug effect. Recognizing the hypothesis as such encourages research on the important questions: To what extent? Under what circumstances? and How?

2) Gonzalez and Byrd point out that if R_d (rate in the presence of the drug) and R_c (rate in the absence of the drug) are related by the equation

$$\log (R_d/R_c) = \log k + j \log R_c$$

then if j is -1, R_d is independent of R_c; that is, the rate after the drug is independent of what the rate would have been if the drug had not been given. They interpret this as meaning that "a regression line with a slope of -1 indicates that the effect of the drug is independent of control rate. . . ." But the *effect* of the drug is surely the *change* due to the drug. If the Internal Revenue Service were to develop an income tax that ensured that everyone had the same income after taxes, I think that the authors would be hard put to convince an erstwhile millionaire that the effect of the tax was independent of income before taxes.

3) Gonzalez and Byrd lay stress on the importance of R_m, the upper limit of response rate, and say that "it is essential to know the value of R_m." They point out, however, that "no study has been conducted in which the value of R_m is either measured or controlled." If R_m were constraining the effect of a drug, we would expect the curves relating drug effect to control rate to bend as they approached an asymptote determined by R_m. No such bending has ever been reported. What has been shown many times is a change in the effect of a drug from rate-enhancing to rate-decreasing at control rates well below R_m. There is evidence that such analyses carried out in ignorance of R_m may be useful beyond their convenience as a means of presenting results (*2*). Indeed, R_m may be not only unknown but, in principle, unknowable; should it be defined as the reciprocal of the shortest interresponse time that occurs, which would impossibly restrict its data base, or should it be defined as a maximum average rate over a finite time, which would mean that there would be instantaneous rates in excess of R_m?

I suppose the moral is that what may be misleading for psychological theory may not be misleading for experimental pharmacology and vice versa. When the first manuscript showing plots of log (R_d/R_c) against log R_c (*3*) was submitted, the question of whether log (R_d/R_c) or log R_d should be ordinate and the fact that with the former method a slope of -1 means that all R_d are the same were discussed with one of the reviewers, D. S. Riggs. The conclusion was that since the two forms of plotting are so readily interconverted it did not matter much, and since the paper was going to a pharmacological journal, whose readers were used to seeing graphs of percentage change due to a drug, it would be best to leave the ordinate as log (R_d/R_c). It still does not seem a matter of great importance, and it would be a pity if Gonzalez and Byrd's concern with the choice of ordinate were to distract readers from more important issues.

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The issue crucial to rate dependency is the form of the relationship between rate of responding in the presence of a drug (R_d) and rate of responding in the absence of the drug (R_c). Dews, however, argues for the appropriateness of plotting proportional change in response rate R_d/R_c as a function of R_c, and states that "the *effect* of the drug is surely the *change* due to the drug." It is obvious that by defining effect as the ratio R_d/R_c, the effect of the drug will of necessity covary with R_c, because R_c is both the variable plotted along the x-axis and the denominator of the ratio plotted along the y-axis. Clearly, the relationship between R_d/R_c and R_c is determined by the form of the relationship between R_d and R_c, in accordance with the rules of algebra. Thus, for example, a regression line with a slope of -1 in a logarithmic plot of R_d/R_c against R_c indicates that R_d is independent of R_c (that is, R_c is not a factor determining R_d). To attribute biological significance to such an inverse relation between R_c and an "effect" defined as R_d/R_c is erroneous and misleading. Dews justifies preference for the ratio R_d/R_c as the dependent variable on the grounds that pharmacologists often express effects as percentage changes. He does not acknowledge that, in such cases, the variable plotted along the x-axis is usually dose or time, or something other than the denominator of the ratio plotted along the y-axis.

Our assertion that the maximum response rate possible (R_m) can affect and limit the outcome of drug experiments and, therefore, should be included in the formulation of general principles of the behavioral effects of drugs is dismissed too expediently by Dews. He indicates that there is no evidence that R_m limits the effects of drugs on response rate and says that R_m may be "in principle, unknowable." Since no attempt has been made to measure or control R_m, his arguments are premature. The absence of reports showing changes in slope as R_m is approached can be ascribed, in our view, to the fact that experimenters have typically imposed straight lines on logarithmic plots of "rate-dependency" data, and accepted deviations from the regression lines as random variability. Furthermore, such plots are not especially effective in revealing constraints due to R_m; other types of data analysis are more appropriate for that purpose. We maintain that R_m can be measured and controlled. The necessary technology has been available for some time, but awareness that R_m could affect the results of experiments in behavioral pharmacology was lacking.

We did not suggest in our article (*1*), nor do we intend to imply here, that there is no empirical support for rate dependency. We maintain, however, that much data purporting to demonstrate rate dependency can be interpreted more appropriately as indicating that drugs cause responding to approach a constant rate. We also believe that the factors identified in our article cannot be ignored when one addresses these issues, and that widely held views about the exact role of R_c as a determinant of the effects of drugs need to be reassessed. We wonder why, after nearly 20 years, rate dependency remains only a hypothesis.

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