presence or absence of a caldera lake is the arrival of the magma body at a crustal level sufficiently shallow to interact with meteoric water. An important factor is the amount of time spent by the magma at shallow crustal levels. Taylor (*1*) has observed that isotopic interactions are most commonly around the margins of intrusions into flat-lying volcanic terranes, but the main control may be merely the shallow crustal positions of the volcanics. This postulated interaction of magmatic bodies of batholithic dimensions with meteoric water, all at liquidus temperatures, has other implications. The water entering the magma may modify the original minor element composition, and this possibility should be considered in studies of strontium and lead in igneous rocks, as well as rocks surrounding large intrusions. The volatiles leaving the magma may carry metal ions that may be the source of ore deposits associated with igneous intrusions. The amount of water available for ore transport by this process is far greater than that available by the simple outgassing of a statically crystallizing magma (even if it is saturated with volatiles).

The course of magmatic differentiation can also be affected by the availability of meteoric water. The accumulation of abundant volatiles in the upper parts of a shallow magma chamber should lower the solidus temperature and the viscosity of the magma in this region, resulting in the establishment of strong physical gradients in this part of the magma. Gradients of this type must have been present in the differentiated magma chamber sources of the Paintbrush and Timber Mountain Tuffs in order to permit the observed variation in phenocryst content, which increases downward in the magma chambers. Finally, convective circulation of water far in excess of that cognate to the magma may have permitted significant differentiation by some volatile transfer process, a differentiation mechanism that has been considered possibly significant (*12*), although the precise nature of such a process is difficult to evaluate.

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Nuclear Magnetic Resonance Studies and Water "Ordering" in the Crystalline Lens

Abstract. *Nuclear magnetic resonance studies of the relaxation times of the water in the crystalline lens show that, as in all interfacial systems, these parameters are markedly reduced from their values in pure water, that* T_2 *is less than* T_1*, and that both depend on water content. Determination of diffusion coefficients and studies on physiologically inert lenses indicate that reduced relaxation times do not provide direct evidence for ordering of the bulk of the cell water.*

Numerous studies of the water of living cells by proton magnetic resonance techniques have appeared in the literature (*1–5*). All of these studies show that both the longitudinal or spin-lattice relaxation time (T_1) and the transverse or spin-spin relaxation time (T_2) are reduced in living cells compared to their values in pure water. Two types of mechanism have been proposed to account for these findings: (i) ordering of the major portion of the cell water (*1*), and (ii) a two-phase rapid exchange mechanism in which only a small fraction of the cell water is ordered (*2, 5*); rapid exchange between this ordered fraction and the bulk water would give rise to an observed relaxation time intermediate between those of the two phases. In an attempt to differentiate between these alternatives, we made measurements of proton relaxation phenomena in the crystalline lens of the eye. Our results give a useful perspective on the entire problem.

We employed pulsed nuclear magnetic resonance (NMR) techniques to examine the relaxation times of the water protons in living frog and rabbit lens in vitro, in various states of hydration and after treatment designed to destroy the physiological integrity of the lens. The diffusion coefficient of the water molecules in rabbit lens was also determined.

For the major portion of the studies on frog lens we used an NMR spectrometer with a 12-inch ($\sim$0.3-m) Varian magnet with flux density 7 kgauss at a frequency of 30.3 Mhz. Time T_1 was determined by using a null method of Carr and Purcell (*6*), and T_2 by using a Carr-Purcell sequence [method B, with pulse train 90°-180°-180° . . . (*6*)]. A few studies of T_2 on frog lens were performed with a Praxis model PR102 with a small permanent magnet and a frequency of 10 Mhz, by using repeated 90°-180° pulse sequences [method A (*6*)]. The term T_2 is usually reserved for the relaxation time of a purely exponential process. Because transverse relaxation is a multicomponent rather than a simple exponential process in the lens (see below), the time for the height of the signal following the 180° pulse to decay to $1/e$ of the height of the 90° pulse will be designated T_2*. Since the T_2* data obtained with the Praxis and the Varian were entirely compatible, they have been pooled.

Studies of rabbit lens were performed with a spin echo apparatus in conjunction with a 12-inch Varian V-4007 research magnet (*7*) at 25.0 Mhz. Time T_1 was determined by using a 180°-90° pulse sequence at varying pulse intervals (*8*), and T_2* was determined by using method A, measuring the voltage with a "boxcar" integrator (*9*), and averaging the results of six repetitions at each pulse interval. This

allowed accurate resolution of transverse relaxation curves to 1 percent of the initial amplitude and showed that transverse relaxation in whole lens as well as nucleus and cortex is actually a multicomponent function. Again, we report only T_2^* from these data. Diffusion coefficients of lens water were measured by using the field gradient spin echo technique as previously described (*6, 10*).

In order to determine the dependence of the relaxation times on the water content of lens tissue, we soaked whole frog lenses for 3 hours in hypoosmotic (120 mosm) and hyperosmotic (480 mosm) Ringer solution before measurement. In addition, relaxation times of the nucleus and cortex, which have quite different water contents, were obtained separately. Measurements were also made on lenses which had been soaked for 3 hours in 5 percent trichloroacetic acid or heated in a microwave oven for 45 seconds. Water content of all lens tissues was determined by weighing before and after drying for 48 hours at 104°C.

Values of T_1 and T_2^* are shown as a function of water content in Fig. 1. The results are similar to those obtained for a number of nonliving interfacial systems, including erythrocyte membranes (*11*), aqueous polymer systems (*8*), and glycerinated muscle (*12*), in the following respects: Both T_1 and T_2^* are significantly reduced from their values in free water, T_2^* is less than T_1, and both relaxation times depend markedly on water content. The first two observations are characteristic of all relaxation times reported for biological material (*1–5*). To our knowledge, this is the first published report of the dependence of relaxation times on water content in living tissue.

The quantitative dependence of T_1 and T_2^* on water content is not consistent with the two-phase rapid exchange model (*13*). However, this does not rule out a two-phase model as the basis of the findings. Exchange between the phases might be slow, or the water content of the tissue might not be uniform. Both cases would give rise to multicomponent curves such as those observed. In addition, the relaxation time of the more ordered fraction may vary with water content if, for example, this fraction is ordered by structural entities whose rate of motion depends on water content.

A number of investigators have suggested that altered solvent properties result from ordering of the major fractions of cell water (*1*). Treatment with 5 percent trichloroacetic acid or with microwaves renders the lens opaque and physiologically inert. Both treatments decreased T_1 and T_2^* without significantly affecting the water content of the tissue (Fig. 1). The significance of this finding is not clear; it might indicate that relaxation times of water protons are influenced by the molecular motion of cellular macromolecules. Coagulation of the tissue by heat or trichloroacetic acid may reduce the motion of the macromolecules, thereby reducing the relaxation times. It is also possible that the interfacial area of macromolecules is increased on denaturation. The finding of decreased relaxation times in these tissues, which show no ability either to exclude or to accumulate solute other than that expected on the basis of a Donnan equilibrium, makes one point clear: It is not possible with our present state of knowledge of macromolecular systems to use NMR relaxation times as direct evidence for altered solvent properties of the water of biological systems.

Table 1. Relaxation times and water diffusion coefficients in rabbit lenses at 36.1°C. Diffusion coefficients (*D*) were obtained as previously described (*6, 10*) by using a pulse spacing of 30 msec for cortex and whole lens and 10 msec for nucleus (*14*).

Sample	T_1 (msec)	T_2^* (msec)	D (cm^2 sec^{-1} $\times 10^{-6}$)	H_2O content (%)
Water	2690†	750†	2.95	100
Whole lens	490	29.3	0.90	65
Cortex	551	40.0	1.06	70
Nucleus	268	7.7	0.97	52

† These are observed relaxation times at 25°C for the apparatus used for rabbit lens. The relaxation times at 36.1°C would be longer. Time T_2^* is shorter than the true T_2 of the system because, with the slow relaxation of pure water, diffusion effects become important. If the T_2 curves are extrapolated to remove this effect, a T_2 value of about 1.6 seconds is obtained. Because of the short relaxation times in the lens samples, diffusion effects are insignificant.

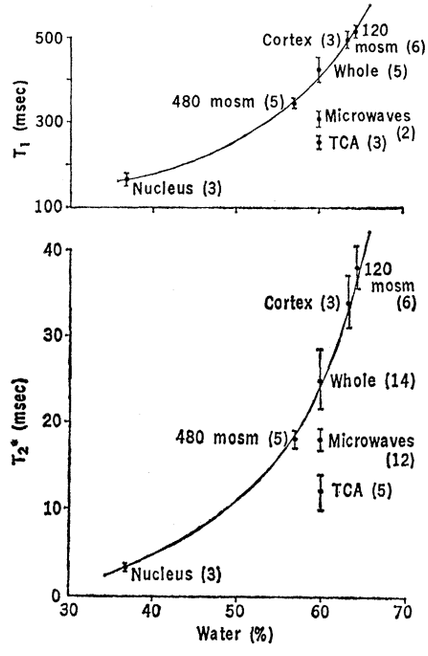

Fig. 1. Values of T_1 and T_2^* in frog lens tissue as a function of water content. The bars represent standard error, and the number of observations is given in parentheses. The standard error of the water content was less than ± 1 percent. Although the water content of lenses treated with trichloroacetic acid (*TCA*) or microwaves is unchanged from that of the whole lens, both T_1 and T_2^* for frog lens are reduced from the values in pure water ($T_1 = 3.03$ seconds and $T_2^* = 1.56$ seconds in pure water).

Throughout this report we have used the word "ordered" without definition—as have most others who wrote on this subject. As Belton *et al.* (*3*) have observed, confusion in this area may have arisen from the assumption that order implies decreased rates of molecular motion. Order may also, however, refer to a statistical distribution of orientations of water dipoles different from that of pure water and

determined by the nature of the interfaces present (*15*). Whether the presence of macromolecules or membranous components in cells brings about more specific or long-range alterations in either the orientation or the motion of the adjacent water molecules is a matter which will require much further study with many more well-defined model systems.

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13. For two phases of water, ordered and normal, when exchange is sufficiently rapid, T ($= T_1$ or T_2) is given by:

$$\frac{1}{T} = \frac{1-F}{T^O} + \frac{F}{T^N} = \frac{1}{T^O} + F\left(\frac{1}{T^N} - \frac{1}{T^O}\right)$$

where F is the fraction of normal water, and T^O and T^N refer to the ordered and normal phases, respectively. A plot of $1/T$ against F should be a straight line if the relaxation time of the ordered water does not change with water content. Such a plot of the data presented in Fig. 1 is not a straight line.
14. Under certain conditions, D can vary when the phase spacing is altered. However, the upper limit for D in lens cortex is the value for pure water. Hence the maximum ratio of D between cortex and nucleus is 3. The ratio of T_2* values is 5.2. This is consistent with our conclusion that T_2* and D are predominantly determined by different water fractions.
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Carbonate Ion–Selective Membrane Electrode

Abstract. *A liquid membrane electrode has been developed with Nernstian response and a high selectivity for carbonate ion. The electrode responds rapidly to carbonate in the 10^{-2}M to 10^{-6}M range with a selectivity for carbonate over chloride, sulfate, and phosphate of at least 10^3 and negligible bicarbonate response. The electrode properties appear to be suited to chemical, biological, and oceanographic measurements.*

Previous attempts (*1*, *2*) to develop bicarbonate ion– or carbonate ion–selective electrodes have resulted in electrodes with poor selectivity for the desired ion with respect to such common interferences as chloride. We have now succeeded in preparing a liquid membrane electrode with a selectivity for carbonate with respect to chloride, sulfate, and phosphate of at least 10^3 and negligible response to bicarbonate. The electrode exhibits Nernstian response to carbonate concentrations in the $10^{-2}M$ to $10^{-6}M$ range (Fig. 1) and has a response time, depending on the concentration levels employed of 30 seconds to 2 minutes.

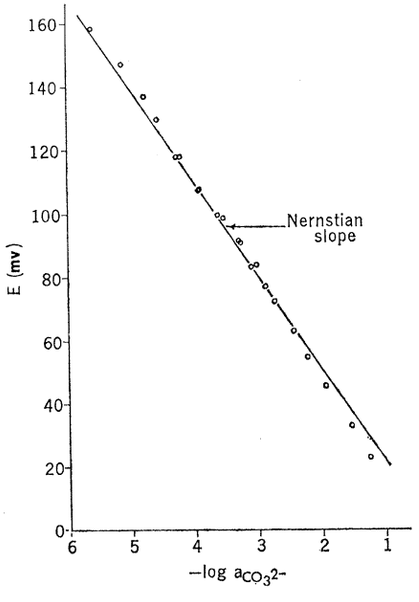

Fig. 1. Response of the electrode to varying carbonate activities. The solid line represents a Nernstian slope; E, electrode potential; a, activity.

The liquid membrane electrode has a conventional construction (*2*), with plastic electrode bodies (Orion) and cellulose acetate support membranes with an average pore diameter of 0.1 μm (Millipore Corporation) or Orion 92-20 membranes. The active electrode material is formed by means of a liquid phase consisting of 1 percent (by volume) of tricaprylyl methylammonium chloride (General Mills Chemicals, Inc., Aliquat 336) dissolved in trifluoroacetyl-*p*-butylbenzene. The latter is prepared by a Friedel-Crafts acetylation (*3*) of butylbenzene with trifluoroacetic anhydride with the use of anhydrous aluminum chloride catalyst, purified by fractional distillation, and identified by mass spectrometry [mass to charge ratio (m/e) = 230], infrared spectroscopy, and nuclear magnetic resonance. The quaternary ammonium salt is used in the chloride form, as received, or may be converted to the bicarbonate form. An aqueous solution, 0.1M in both sodium chloride and sodium bicarbonate, is used as the internal electrode reference solution. All measurements are taken against a double-junction reference electrode (Orion).

Since carbonate coexists with bicarbonate in *p*H-dependent equilibrium, the carbonate concentrations for Fig. 1 are calculated from the relevant equilibrium constants (*4*) ($K_1 = 4.5 \times 10^{-7}$, $K_2 = 4.8 \times 10^{-11}$) and the modified Davies equation (*5*). The solid curve represents the theoretical Nernstian slope for a divalent anion, and the experimental points represent