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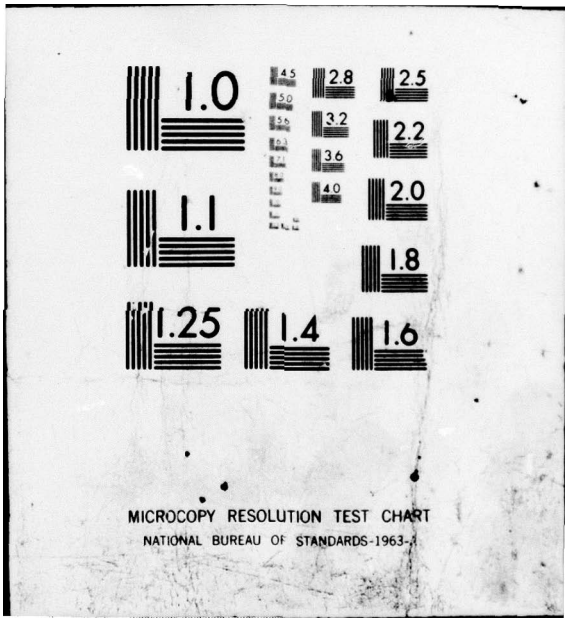
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Diffusion measurements in three types of frog muscle have yielded a constant value of 1.7 X 10^{-5} cm^2/sec at the shortest times, indicating that the intracellular material does not have a high microviscosity, nor does it contain effective diffusion barriers. The diffusion coefficients at longer times indicate an outer membrane permeability of 0.01 cm/sec. This work has been accepted in Biophysical Journal.

Diffusion measurements of water in packed red cells yielded an intracellular diffusion coefficient (D_o) of 6 x 10^{-6} cm^2/sec, indicating a much higher microviscosity than is the case in frog muscle. The permeability of 0.01 cm/sec is in good agreement with that obtained by osmotic shock and by NMR spin-relaxation methods. A portion of this work was presented at ACS Symposium Series No. 34 (1976). Measurements of water diffusion within E. coli, yeast, a fresh water algae, nuclei of calf brain cells, epidermal and subcutaneous fat layers of rhesus monkey skin yielded intracellular diffusion coefficients ranging from 5 x 10^{-6} to 1.4 x 10^{-5} cm^2/sec. Much lower values were attained for diffusion of water in partially hydrated brine shrimp. For nearly all of the samples the measurement will also permit the calculation of the cell membrane permeabilities to water. The diffusion coefficient of oil in human adipose tissue was found to be 2 x 10^{-7} cm^2/sec.

The unpublished portions of this work are being prepared for publication in the open literature.

As an aid in interpreting the data, a general treatment of transient diffusion in one dimension between regularly spaced permeable barriers in a medium of finite viscosity has been performed. The results at short and long time limits are as expected, except for an unexplained dependence of the theoretical diffusion coefficient on the strength of the field gradient assumed to be used in the measurements. This work has been published in J. Chem. Phys. 69 1748 (1978).

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The object of this program was to measure rates of self diffusion of water and other molecules within and between cells, and to use the results to infer intracellular viscosity, structure, and/or absorption, and to determine permeabilities of the cell outer membrane.

The pulsed field gradient nuclear magnetic resonance method was selected. Accessories allowing the attainment of diffusion times between 0.3 msec and several seconds were constructed and used in conjunction with conventional pulse NMR

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PREFACE

Grateful thanks are due to the following faculty of Indiana University and their students for furnishing samples, for permitting use of their facilities for further sample preparation and handling, and for helpful discussions: Prof. Arthur L. Koch, Department of Microbiology, Profs. Al Strickholm and Rey Elizondo of the Department of Physiology, and Prof. Henry Mahler, Dr. James McDonough, Mr. Kerry Blanchard and Ms. Margaret Loyd of the Department of Chemistry.

Samples were also furnished by the laboratory of the Bloomington Hospital, Bloomington, Indiana and by Dr. Patty Seitz of the University of Texas, Medical Branch, Galveston, Texas.

The algae was cultured by Ms. Kathy Andrews of the Naval Weapons Support Center, Crane, Indiana.

A special thanks are due to Prof. Arthur Clouse and his assistants Mr. Bob Addleman and Mr. Deon Osman of the Department of Chemistry of Indiana University for modifying their NMR apparatus especially for the performance of these experiments.

INTRODUCTION

There is presently considerable interest in the mobilities of molecular species within cells and through cell membranes. Knowledge of these quantities allows a better understanding of the limiting steps of biochemical reactions and, more specifically, allows estimates of limits to the thermodynamic efficiencies of these reactions. In this project we have selected the pulsed field gradient nuclear magnetic resonance method[1] to measure diffusion of water and a few other substances in a wide variety of cellular systems.

Magnetic field gradient NMR is well adapted to measuring diffusion in colloidal systems, including biological cells, because the experimental measurement times by this method are such that the distances traveled by the molecules are of the same order as the dimensions of the inhomogeneities of the system. The result is that the apparent diffusion coefficients are dependent on the diffusion time. By varying this latter parameter the dimensions of the inhomogeneities as well as the local diffusion coefficients within them can be obtained.[2]

The maximum information is obtained by the use of the widest possible range of diffusion times. In the work reported here, a variety of recently developed field gradient NMR techniques have been used to get a much wider range of diffusion times than have been employed in previous studies of diffusion in biological materials.

EQUIPMENT

The theory of the use of pulsed field gradient NMR to measure intracellular self diffusion has been outlined in Annual Report No. 1[3] of this project. The equipment necessary for performing such experiments has been assembled, and a description was included in that report.

Briefly, a Varian DP-60 system was modified for high power rf pulses. A frequency modulated external fluorine lock provided field-frequency stability. A current source and probe coils were constructed capable of producing field gradient pulses of either sign, up to 600 G/cm, and of duration up to several milliseconds. By the use of alternating sign pulse trains[4], diffusion times as low as 0.3 msec were obtained, while the stimulated echo method[5] allowed diffusion times as long as 2.5 sec.

THEORETICAL DEVELOPMENT

In order to interpret the data therotical functions of the time-dependence of diffusion coefficients in heterogeneous systems were needed. Until this time there has been no derivation reported for a case of diffusion in regularly spaced barriers of arbitrary permeability immersed in a medium of finite viscosity, such as exists in a cellular system. Therefore a general treatment of the one-dimensional case was performed.

The essentials of the results are contained in a previous report,[6] and were presented as poster W-POS-K2 at the March 1978 joint meeting of the Biophysical Society and the American Physical Society in Washington D. C. A complete report has been written and has appeared in J. Chem. Phys. 69, 1748 (1978), entitled "Transient Diffusion in a System Partitioned by Permeable Barriers. Application to NMR Measurements with a Pulsed Field Gradient". The results for the limiting cases agreed with expectations, except for a puzzling dependence of the predicted diffusion coefficient on the assumed field gradient, even at long diffusion times.

EXPERIMENTAL RESULTS

Diffusion measurements in three types of frog muscle have yielded a constant value of 1.7×10^{-5} cm^2/sec at the shortest times, indicating that the intracellular material does not have a high microviscosity, nor does it contain effective diffusion barriers. The diffusion coefficients at longer times indicate an outer membrane permeability of 0.01 cm/sec for two of the types and 0.04 cm/sec for the third type. These measurements are discussed in an earlier report[7], and have been accepted for publication in Biophysical Journal, along with a review of other NMR measurements of diffusion in muscle.

Diffusion measurements of water in packed red cells yielded an intracellular diffusion coefficient (D_0) of 6×10^{-6} cm^2/sec, indicating a much higher microviscosity than is the case in frog muscle. The permeability of 0.01 cm/sec is in good agreement with that obtained by osmotic shock and by NMR spin-relaxation methods. These measurements have been reported more fully earlier[7], and a brief presentation is also contained in reference 2b.

Diffusion measurements have also been performed on a sample treated with glutaraldehyde, so as to crosslink and denature the membrane protein. The object was to determine whether protein "pores" contribute significantly to the membrane permeability for water.

An increase in diffusion coefficients over the entire range of diffusion times was found. It is speculated that this was caused by a swelling of the cells. Attempts to confirm this swelling by other methods were inconclusive. The decrease in diffusion coefficients at longer times indicated the membrane is still a barrier to water after the glutaraldehyde treatment, but unfortunately the measurements were not carried to long enough diffusion times to determine whether the permeability had changed.

The proton signal of hemoglobin in a D_2O-exchanged sample was observed; however reliable diffusion measurements could not be obtained because of the low signal strength caused by the short T_2, about 1 msec, and because of interference from residual HDO. However with larger diameter sample tubes an accurate measurement should be possible.

Diffusion measurements in a packed sample of E. coli yielded a lower limit of $p > 0.1$ cm/sec for the permeability of the outer membrane to water. This is much higher than for the muscle and red cells. The lower limit on D_0 is 6×10^{-6} cm^2/sec. The results were restricted to limits rather than actual values because of the small cell size in

combination with the high membrane permeability. The results are contained in an earlier report[7].

Measurements on other systems have recently been performed. The data are still being worked up and analyzed, however some of the results can be stated:

A large number of samples of centrifuged or filtered pellets of yeast, a haploid variety of cell diameter about 3 μm, were studied. It has been possible to distinguish a large amount of extracellular water apparently trapped in the cell walls -- all of the cultures were allowed to grow to maturity. The intracellular diffusion coefficient is 5×10^{-6} cm^2/sec. The data are sufficient and accurate enough to allow calculation of the extra-cellular water diffusion, as well as of the membrane permeability.

Measurements on a fresh water alga, Chlorella vulgaris yielded D (intracellular) $\simeq 5 \times 10^{-6}$ cm^2/sec. The data will also allow calculation of the membrane permeability as a check on an earlier measurement by an NMR spin-relaxation method[8].

Intranuclear water was found to have a self diffusion coefficient of 1.4×10^{-5} cm^2/sec in a pellet of freshly prepared nuclei of calf brain cells. This is much higher than that for the single cell organisms, and is approximately equal to the value found in muscle cells. A nuclear membrane permeability of 0.01 cm/sec was found.

An intracellular diffusion coefficient of 2×10^{-7} cm^2/sec was found for the major mobile protonated component in a sample of healthy adipose tissue obtained from a human breast biopsy. A slight dependence on the diffusion time is being analyzed in terms of droplet and cell sizes. Similar values were obtained from a less detailed study of subcutaneous fat in rhesus monkey.

The diffusion coefficient of intracellular water perpendicular to the plane of the epidermis of rhesus monkey (taken from the palms) was found to be 7×10^{-6} cm^2/sec. The time-dependence is being analyzed. A similar value was obtained for water in the subcutaneous fat sample mentioned above.

Measurements in samples of brine shrimp, Artemia salina, at three different degrees of hyrdation showed two water components, presumably extra cellular and intracellular. An intracellular diffusion coefficient of $1. \times 10^{-7}$ cm^2/sec was found for the least hydrated sample, which was 0.12 g water/g dry weight. Calculations for the other samples are in progress. All samples were shown to be viable at the end of the measurements.

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