tion of the sodium gating by this formalism involves the numerical integration of many more simultaneous equations than do other models used to describe this process. The need for the extra equations comes directly from the hypothesis of nonequilibrium dielectric polarization, the need for which hypothesis in turn comes directly from the observation of the τ_c-τ_h separation.

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HIGH-FREQUENCY DIELECTRIC SPECTROSCOPY OF CONCENTRATED MEMBRANE SUSPENSIONS

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The interfaces between biological membranes and their aqueous environments are of special interest to biology. This is because the many substances that bind to membranes, react with membranes, or pass through membranes must first enter and interact with these interfaces. We investigated the physiological chemistry and physics of the membrane-aqueous interface by high-frequency dielectric spectroscopy of concentrated suspensions of membranes. We adapted the recently developed "time-domain" technique for dielectric measurements (1). We observed a previously unreported dielectric absorption in concentrated suspensions of membranes obtained from either the outer segments of rod photoreceptor cells of the retina or blood erythrocytes.

We observed an anomalous dielectric absorption in these membranes having a dielectric constant of about 1,000 and a characteristic frequency, f_c, of about 170 MHz. It is a discreet absorption, well separated from the closest characteristic frequencies, 1 and 20,000 MHz, previously reported in tissue or cell suspensions (2). The discreet nature of the absorption ruled out the possibility that this absorption is the tail end of one of the neighboring absorptions. It also ruled out an artifact of the conductivity contribution to dielectric loss (3).

The anomalous dielectric absorption was not observed in suspensions of erythrocyte ghosts as dilute as 1:10. It became progressively stronger as the concentration of membranes increased, and grew to a dielectric constant of about 1,000 with centrifugally packed suspensions of erythrocyte ghosts or rod outer segments. As more aqueous solution was removed from the membrane suspension, the dielectric constant dropped. Thus, with rod outer segments the dielectric constant demonstrated a maximum value at about 60% water (wt/wt), corresponding to the reported values of water content of rod outer segments in vivo. The anomalous dielectric constant of membrane suspensions varied directly with conductivity as well as with the sodium chloride content of the aqueous solution.

We investigated the temperature dependence of the anomalous dielectric constant in erythrocyte ghost suspensions. Plotted against $1/T$, the logarithm of the dielectric constant produced a straight line. The dielectric constant increased by a factor of 1.6 as the temperature increased from 4° to 36°C. This dependence was opposite to the temperature dependence of dielectric constants exhibited by dipole orientation polarizations, and it was much larger than that exhibited by Maxwell-Wagner polarizations (4). The characteristic frequency of 170 MHz, together with the temperature dependence of the dielectric constant, ruled out the Maxwell-Wagner mechanism for this polarization. The temperature dependence of the anomalous dielectric constant had the same sign and approximate magnitude as the temperature dependence of ionic conductivity. Although we showed strong correlation of dielectric constant with conductivity, previous conductivity based dielectric mechanisms have been relevant only to much lower frequencies, i.e., about 10^4 Hz (5).

The value of 170 MHz for f_c corresponds to the characteristic frequency attributed to the tightly bound first "monolayer" of water of hydration on protein (6). This correspondence, along with its dependence upon water concentration, suggests that the anomalous dielectric absorption is due to water of hydration. Its additional dependence upon salt concentration and conductivity indicates that the anomalous dielectric absorption depends upon mutual or collective interactions of the membranes, water, and ions.

The characteristic frequency of aged erythrocyte ghosts or those prepared from outdated donor blood was higher by a factor of 1.5 than the f_c of fresh membranes. Consequently, we can correlate changes in the dielectric properties with changes in structure (7) and function known to occur with age in erythrocyte membranes. Furthermore, the age dependence of the characteristic frequency suggests that dielectric measurements are relevant to membranes that constantly regenerate, e.g., photoreceptor membranes and erythrocytes.

We observed this new dielectric absorption with a variety of different types of membrane preparations, viz., erythrocytes, erythrocyte ghosts, rod outer segments, isolated photoreceptor membranes, and photoreceptor membrane vesicles. Apparently, this dielectric absorption is a general phenomenon occurring with membranes. It may have been overlooked previously because it becomes noticeable only at high cell or membrane concentrations, because of interference by the enormous Maxwell-Wagner

polarization, and because of the difficulty that the high conductivity of biological samples causes with dielectric measurements.

A dielectric polarization process with a large dielectric constant associated with the aqueous environment of membranes may exert a dominant effect upon the mobilities of ions and charged functional groups near the membrane surfaces. Consequently, the dielectric process that we observed may exert a major effect upon both the structure and function of biological membranes. We can now interpret these physical measurements in terms of biochemical properties of membranes. We have shown that high-frequency dielectric measurements are physiologically relevant to active membrane systems that constantly regenerate, especially if they are closely spaced in vivo, e.g., photoreceptor membranes in rod outer segments of the retina.

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SUBNANOSECOND FLUORESCENCE LIFETIMES BY TIME-CORRELATED SINGLE PHOTON COUNTING USING SYNCHRONOUSLY PUMPED DYE LASER EXCITATION

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The measurement of fluorescence parameters, particularly fluorescence lifetimes, represents one of the principal techniques for studying the photophysical properties of organic molecules and for elucidating the dynamic chemical and physical processes seminally important in molecular biology. Particularly informative are changes in lifetimes with different environmental factors that mimic physiological states. Time-correlated single-photon counting has been applied to nanosecond fluorescence measurements since the mid-1960s. Because of the inherent characteristics of the traditional excitation source, the air gap-discharge arc, there are major problems associated with lifetime measurements when fluorescence intensities are low. Recently a syn-