LETTERS TO THE EDITOR

$^{23}Na^+$ Interaction with Bacterial Surfaces: a Comment on Nuclear Magnetic Resonance Invisible Signals

Dear Sir:

Magnetic resonances of quadrupolar nuclei have been used extensively in probing biological systems (1). Early attempts at studying $^{23}Na^+$ in tissue, such as frog muscle (2), demonstrated that only a fraction of the total signal expected could be found in the nuclear magnetic resonance (NMR) experiment. This was originally attributed to loss of signal from Na^+ which was bound. Recent experiments, however, have demonstrated that 60% of the signal from $^{23}Na^+$ is found in satellites, which may be too broad to be observed, when this quadrupolar nucleus is in an anisotropic environment (3–6). Civan and Shporer (7), in light of recent explanations, have examined the ^{17}O resonance of water in frog muscle and observed only 75% of the maximum signal expected from all the ^{17}O present. Two possible explanations were proposed. There is either a mobile and immobile state of water, or approximately 33% of the total water is in an anisotropic medium.

In our laboratories we have been examining the binding of ions to membranes (8) and bacterial cell surfaces (9). The line width of ^{23}Na resonances, observed at 15.8 MHz, can be used to detect interaction of the ions with the membranes or cell surfaces. Under simplifying conditions of rapid exchange, one can assume that the line width observed, $\Delta\nu_{obs}$, is a weighted average of those representing all species.

$$\Delta\nu_{obs} = \Delta\nu_f X_f + \sum_i \Delta\nu_{bi} X_{bi} .$$

The line widths of the bound and free species are represented by $\Delta\nu_b$ and $\Delta\nu_f$, respectively, and the X's refer to respective fractions. If X_f is close to 1.0, then $\Delta\nu_f X_f$ can be represented by the line width of the standard 0.16 M NaCl, $\Delta\nu_{std}$. Further, if one replaces the summation over all sites i by an average line width, $\Delta\nu_{ave}$, then one obtains the following:

$$\Delta\nu_{obs} - \Delta\nu_{std} = \Delta\nu_{ave} X_b .$$

As a measure of X_b we have used the dry weight of the bacteria, and this is the abscissa in Figs. 1 and 2.

This approach predicts a straight line through the origin for a plot of $\Delta\nu_{obs} - \Delta\nu_{std}$ vs. dry weight of bacteria. Fig. 1 presents the results obtained with *Sarcina ureae*. Also plotted in this figure is the percent of maximum expected intensity of the ^{23}Na resonance. The total signal is accounted in the observed resonance. Intensity was determined by integration and comparison with standards. Fig. 2 shows the results for *Pseudomonas aeruginosa*. In this case the line width plot approximates a straight line but it does not pass through the origin. The intensity plot shows that at high concentrations only 40% of the maximum expected intensity is observed. This corresponds to the intensity expected for a quadrupolar nucleus in an anisotropic medium. We have not succeeded in observing any satellites. That the line width plot in Fig. 2 does not pass through the origin indicates the simplified treatment above is not complete.

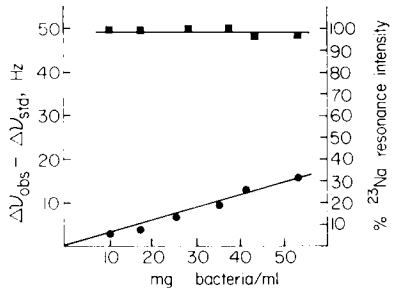

FIGURE 1

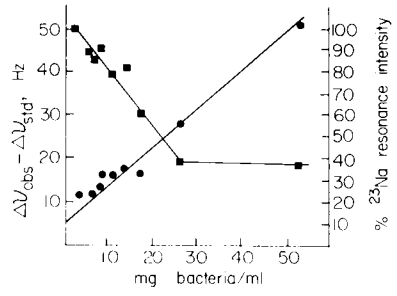

FIGURE 2

FIGURE 1 Effect on ^{23}Na resonance of varying concentrations of *S. ureae* in 0.16 M NaCl. Line width, -●—●-, vs. dry weight of bacteria per milliliter; -■—■-, percent of maximum intensity vs. dry weight of bacteria per milliliter.

FIGURE 2 Effect on ^{23}Na resonance of varying concentrations of *P. aeruginosa* in 0.16 M NaCl. Line width, -●—●-, vs. dry weight of bacteria per milliliter; -■—■-, percent of maximum intensity vs. dry weight of bacteria per milliliter.

Both results obviously must reflect differences in surface structure of the bacteria. *P. aeruginosa* is gram-negative and *S. ureae* is gram-positive. The cell envelopes of gram-positive and gram-negative bacteria are known to differ in composition and structure (10). Note that the line broadening is much greater for the gram-negative than for the gram-positive species. We have compared over 20 species, and this generalization appears to be very good. Besides a smaller effect on line width, the *S. ureae* did not reduce signal intensity even at higher concentrations.

Berendson and Edzes (6) have suggested that a dispersion of cell membranes could lead to loss of intensity. The medium is anisotropic since ions will experience different fields over time periods related to diffusion throughout the liquid phase. Satellite signals corresponding to 60% of the total signal need not be visible. Civan and Shporer (7) have explained loss of signal intensity in terms of immobilization or compartmentalization where compartment boundaries need not be rigid barriers. Our results strongly support this latter possibility where the compartment is defined as an anisotropic region through which the ion diffuses. The anisotropic region is near the bacterial surface and obviously is determined by the properties of that surface.

The requirement for a broad and narrow component, the unobserved and observed signals, respectively, is that structural heterogeneity exists over distances of at least 100 Å. This condition most certainly holds in bacteria dispersions. At high concentrations of bacteria, all the sodium experiences an average field gradient fluctuating at a rate less than the resonance frequency. A signal representing 40% of the total signal is obtained. At lower bacteria concentrations, a portion of the Na^+ experiences the same condition, but another portion, with 100% of its amplitude in a narrow resonance at the position of the 40% resonance from the other fraction, experiences an average field with fluctuations at rates much greater than the resonance frequency. These fluctuations are produced when ions diffuse through the different electric field gradients established by the surface of the bacteria cell envelopes.

It has been shown (6, 11) that relaxation of an exchanging $I = 3/2$ nuclei, must be described by several exponential contributions. Deviation from linearity in Fig. 2 may reflect different combinations of these contributions. A more complete description of the relaxation

phenomena will be obtained in further work with pulsed NMR studies which enable measurements of the relaxation times.

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The Double Fixed Charge Membrane Model: an Hypothesis Concerning the Structure and Morphogenesis of Cell Membranes

Dear Sir:

The electrical properties of a membrane containing two fixed charge regions, of opposite sign, in juxtaposition, show many similarities to those of cell membranes (e.g., Coster, 1965, 1969, 1973 *a*, *b*). In the theoretical analysis of this membrane model previously given (e.g., Mauro, 1962; Coster, 1965, 1973 *a*, *b*), neither the question of a possible physical realization of the model system, nor the relationship between the model and the structures postulated for the cell membrane, was considered.

Based on the hypothesis of the presence of a double fixed charge system in cell membranes, we now wish to put forward an hypothesis of the molecular organization, morphogenesis, and appearance, under electron microscopy, of the cell membrane.

Proteins, either as a separate phase or in various lipoprotein and glycoprotein complexes are a major constituent of all cell membranes. For the present discussion they are, as suggested by much recent work (e.g., see Fox, 1972), assumed to be distributed throughout the bulk of the membrane. Fixed charges in the cell membrane could arise on the membrane