LETTER TO THE EDITOR

# *Nuclear Magnetic Resonance of $^{23}Na$ in Suspensions of Pig Erythrocyte Ghosts: A Comment on the Interpretation of Tissue $^{23}Na$ Signals*

Dear Sir:

In preceding papers (1, 2), one of us presented an interpretation of the nuclear magnetic resonance (NMR) signal of $^{23}Na$ in biological tissue. According to this interpretation, the peculiar magnetic behavior of $^{23}Na$ nuclei in tissue (i.e., reduced resonance intensity, two different transverse relaxation times $T_2$, and a single longitudinal relaxation time $T_1$ [3–7; other references can be found in ref. 1]) can be attributed to the nuclear quadrupole interactions of a fraction of tissue $^{23}Na$ and electric field gradients in the neighborhood of (or within) subcellular membranes (or other components of the particulate fractions); and this fraction of tissue $^{23}Na$ ($^{23}Na$ in state B) will be in rapid exchange with the remaining tissue $^{23}Na$, which is characterized by a short correlation time ($\ll$ the Larmor period) and a single Larmor frequency. $^{23}Na$ in state B will possess, in the absence of exchange, (*i*) two different $T_2$ and/or (*ii*) more than one Larmor frequencies in the first order perturbation effect.

It is well known that the time of exchange of $Na^+$ across the cell membrane is far longer than $T_2$ of $^{23}Na$ in NaCl solution (57 ms for dilute solution [8]). Accordingly, as pointed out before (2), if $^{23}Na$ in state B is absent on (or in the neighborhood of) the outer surface of the cell membrane (i.e., if the outer surface of the cell membrane does not possess the ability to depress the resonance intensity of $^{23}Na$), the observed signal of tissue $^{23}Na$ is quite difficult to explain by the current interpretation.

However, Jardetzky and Wertz (9) reported that the resonance intensity of $^{23}Na$ in whole dog blood, erythrocyte ghost suspension in saline, and other samples containing the cell membrane of erythrocytes is identical to that of physiological saline. In a more recent paper, Yeh et al. (10) concluded that no "NMR-invisible sodium" occurs in packed human erythrocytes and its hemolyzate. These results seem to render unlikely the occurrence of $^{23}Na$ in state B in the cell membrane.

We carefully reexamined this point and found that erythrocyte ghosts possess the ability to depress the resonance intensity of $^{23}Na$ (Table I). Adult pig blood was collected into 1/10 vol of 0.1 M ethylenediaminetetraacetic acid (EDTA) neutralized with NaOH. All further procedures were performed at 1–3°C. The erythrocytes were washed three times with 6 vol of 150 mM NaCl containing 1 mM EDTA neutralized with NaOH by centrifugation at 200 *g* for 5 min. The packed cells were hemolyzed by the addition of 10 vol of 14 mM Tris-1 mM maleate-HCl buffer containing 1 mM EDTA, pH 8.2–8.4 (solution H). The erythrocyte ghosts were washed five times with 5 vol of solution H by centrifugation at 20,000 *g* for 25 min. A small pink button at the bottom of the centrifuge tube was discarded. The ghosts were further washed twice with 4 vol of 14 mM Tris-3.5 mM maleate-HCl buffer, pH 6.8–7.0, by centrifugation at 20,000 *g* for 25 min. The ghosts were further packed by centrifugation at 75,000 *g* for 60 min. To 1,620 mg and 810 mg aliquots of the packed ghosts were added 180 mg and 990 mg, respectively, of maleate-NaCl-NaOH buffer, pH 6.8–7.0. To other 1,620 mg aliquots were added 180 mg of maleate-NaCl-NaOH buffer containing guanidine hydrochloride (G-HCl), pH 6.8–7.0. The final concentrations of $Na^+$ and maleate in the 1.8 g samples were 138 mmol/kg sample and about 10 mmol/kg sample, respectively. The NMR signal (derivative of absorption mode) of $^{23}Na$ in the 1.8 g samples was obtained at 22–24°C with a wide-line spectrometer

TABLE I

RESONANCE INTENSITY OF $^{23}$Na IN SUSPENSIONS OF PIG ERYTHROCYTE GHOSTS

| Ghost concn. | Resonance intensity | Loss in intensity per 1% ghosts |
|---|---|---|
| *% as dry wt* | % | %/% |
| 2.48 | 85.5 ± 1.0 | 5.8 ± 0.4 |
| 4.95 | 73.3 ± 1.2 | 5.4 ± 0.3 |
| 4.95 (+ G-HCl, 0.6 mol/kg sample) | 98.7 ± 1.0 | ~ 0.3 ± 0.2 |

The resonance intensity of $^{23}$Na is expressed in percentage of the intensity expected from the Na content. The value after a ± sign is the SEM of five samples. G-HCl is guanidine hydrochloride.

(Varian Associates, Palo Alto, Calif.; model V-4200B with a V-3606 electromagnet) in the same manner as described in a preceding paper (1). A relatively large amplitude of field modulation was used. This gave practically identical line widths to the signals of all the samples examined. The erythrocyte ghosts were practically hemoglobin free. With phase-contrast microscopy, they were markedly shrunk and almost all of them were in the forms of crenated spheres and crenated discs; no fragmentation was observed. The results are shown in Table I.

The ability (per unit dry wt) of erythrocyte ghosts to depress resonance intensity of $^{23}$Na was roughly twice as large as that of the whole homogenate of rat liver. In the presence of guanidinium ion (600 mmol/kg sample), the resonance intensity of $^{23}$Na was restored to near the 100% level; this suggests that the observed loss in the resonance intensity of $^{23}$Na in ghost suspensions does not arise as an artifact.

The present result disagrees with that of Jardetzky and Wertz (9). But in their short report the concentration of ghosts was not specified. In dilute suspensions of ghosts (1% or less as dry wt) and also in whole blood and packed erythrocytes, the loss in the resonance intensity of $^{23}$Na was barely detectable.

Of course, the present result does not necessarily imply that the outer surface of the cell membrane possesses the ability to depress the resonance intensity of $^{23}$Na. Lindblom (11) observed, however, a quadrupole splitting of the $^{23}$Na signal in lamellar liquid crystals of lecithin (64% by wt)-sodium cholate (16%)-water (20%). This observation suggests that the ability to depress the resonance intensity of $^{23}$Na is widely distributed among subcellular membranes, which contain, as main constituents, phospholipids and other molecules with anionic groups. In fact, this ability is found in mitochondrial (900 $g$ × 10 min to 8,000 $g$ × 10 min), heavy microsomal (8,000 $g$ × 10 min to 20,000 $g$ × 40 min), and light microsomal (20,000 $g$ × 40 min to 100,000 $g$ × 60 min) fractions of rat liver homogenate (10% homogenate in 0.25 M sucrose); and the abilities (per unit dry wt) of these subcellular fractions are some 1.5–2 times larger than that of the whole liver homogenate (unpublished result). Since both surfaces of the cell membrane also possess anionic groups of phospholipids and other molecules, it is reasonable to assume that $^{23}$Na in state B occurs on the outer surface of the cell membrane and on its inner surface as well.

In summary, (*a*) pig erythrocyte ghosts possess the ability to depress the resonance intensity of $^{23}$Na; (*b*) available evidences and discussion suggest that both surfaces of the cell membrane possess this ability; and (*c*) accordingly, a difficulty in interpreting the tissue $^{23}$Na signal seems overcome. The effect of guanidinium ion on the resonance intensity of $^{23}$Na in suspensions of pig erythrocyte ghosts was also reported.

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