tergent, consisting of two parts with opposite properties, an N-terminal hydrophilic "handle" with three residues positively charged at physiological pH and two juxtapositioned half-cystine residues, adjacent to a part with extraordinary hydrophobicity. Presumably, these properties account for the vital surfactant activity of the polypeptide in the lung.

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Uptake of Cesium Ions by Human Erythrocytes and Perfused Rat Heart: A Cesium-133 NMR Study

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ABSTRACT: Cesium-133 NMR studies have been carried out on suspended human erythrocytes and on perfused rat hearts in media containing CsCl. The resulting spectra exhibit two sharp resonances, arising from intra- and extracellular Cs^+, separated in chemical shift by 1.0–1.4 ppm. Thus, intra- and extracellular resonances are easily resolved without the addition of paramagnetic shift reagents required to resolve resonances of the other alkali metal ions. Spin–lattice relaxation times in all cases are monoexponential and significantly shorter (3–4 times) for the intracellular component. When corrections are made for the pulse repetition rate, the total intensity of the intracellular and extracellular Cs^+ resonances in erythrocytes is conserved, implying total observability of the intracellular pool. The uptake of Cs^+ by erythrocytes occurs at approximately one-third the reported rate for K^+ and was reduced by a factor of 2 upon addition of ouabain to the sample. These results indicate that ^{133}Cs NMR is a promising tool for studying the distribution and transport of cesium ions in biological systems and, in some cases such as uptake by cellular Na,K-ATPase, for analysis of K^+ ion metabolism.

Interest in the biochemistry and physiology of cesium ions derives primarily from three areas: (1) applications related to elucidating the general properties of alkali metal ion transport and enzyme activation (Eisenman & Krasne, 1975), (2) toxicologic problems related to the uptake and passage through food chains of radioactive Cs^+ produced in fission

reactions (Sastry & Spalding, 1968), and (3) applications of Cs^+ as a pharmacologic agent in the treatment of behavioral depression (Messiha, 1978).

In those systems studied, Cs^+ activates Na/K-dependent ATPases in the absence of K^+ (Skou, 1960; Whittam & Ager, 1964; Bader & Sen, 1966; Baker et al., 1969) and is transported into cells via pathways sensitive to ouabain inhibition (Love & Burch, 1953; Beauge & Sjodin, 1968). The concentration of Cs^+ required for activation of Na/K-ATPase and the transport rates vary depending on the system studied; generally Cs^+ is less effective than Rb^+ as a substitute for K^+. In preparations with excitable membranes, Cs^+ blocks voltage-dependent channels that normally conduct Na^+ or K^+ ions, whereas the resting permeability of Cs^+ (as measured by tracer fluxes) is 0.3–0.1 times the K^+ permeability (Mullins, 1975). From a toxicological standpoint, the facile assimilation of Cs^+ by plants and animals, coupled with its efficient transfer through the food chain to humans and deposition in muscle and other soft tissue, makes an understanding of Cs^+ biochemistry of central importance in the development of strategies designed to increase the excretion and decrease the retention of radioactive Cs^+ (Sastry & Spalding, 1968). Equally important is an assessment of the distribution of Cs^+ in organisms administered doses likely to be used for therapeutic purposes (Krulik et al., 1980).

Recent in vivo NMR studies of the physiology of $^7Li^+$, $^{23}Na^+$, and $^{39}K^+$ have been stimulated by the development of aqueous, paramagnetic shift reagents that induce changes in the resonance frequencies of the alkali metal ions that are in excess of their line widths, thus enabling resonances of the intra- and extracellular cation pools to be resolved (Pike & Springer, 1982; Gupta & Gupta, 1982; Adam et al., 1987; Pettegrew et al., 1987). It should be noted, however, that difficulties exist in the use of such reagents in studies of biological systems, most notably the competitive binding of extracellular divalent ions, particularly calcium, by the chelators (Gullans et al., 1985), as well as the undesirable physiologic side effects produced when lanthanide ions are released as a consequence of the degradation of some of these reagents (Matwiyoff et al., 1986).

In our initial considerations of the feasibility of carrying out in vivo NMR studies of $^{133}Cs^+$, we noted that the small quadrupolar moment of the 100% abundant ^{133}Cs isotope, coupled with the high sensitivity of the Cs^+ chemical shift to the nature and concentrations of counterions in solution (Halliday et al., 1969), could lead to conditions under which the intra- and extracellular Cs^+ pools are resolved without introducing extracellular shift reagents. As demonstrated in the present study, this turns out to be the case, both in studies of suspended human erythrocytes and in the perfused rat heart. Hence, in vivo Cs^+ NMR studies are readily performed and, in situations such as the assay of the activity of Na/K-ATPase in which Cs^+ ions have been proven to behave in a manner analogous to that of K^+ ions, Cs NMR may represent an alternative assay method of choice.

Experimental Procedures

Red Blood Cell Preparation. Freshly drawn, heparinized, human blood was washed twice in cold isotonic saline solution (0.85% w/v NaCl). The lightly packed cells were resuspended at a hematocrit of approximately 40% in a solution containing 140 mM NaCl, 10 mM CsCl, and 5 mM glucose, and approximately 2 mL of this suspension was transferred to a 10-mm o.d. NMR tube. If the blood sample was not used immediately for NMR experiments, the washed cells were resuspended in a buffer containing 145 mM NaCl, 5 mM KCl, 5 mM glucose, and 10 mM Tris/Hepes[1] buffer and stored at 4 °C, generally for 8–16 h. Before these cells were used, they were resuspended at a 10% hematocrit in fresh K^+ buffer and incubated at 37 °C for approximately 1 h. After incubation, the K^+ buffer was removed and the cells were resuspended at the higher hematocrit in the 10 mM Cs^+ "buffer".

Heart Perfusion. Adult male Sprague-Dawley rats (Charles River Suppliers, Wilmington, MA) weighing between 220 and 300 g were anesthetized with pentobarbital. The heart was excised and the aorta cannulated within 15 s. Retrograde perfusion was instituted from a reservoir 80 cm above the aortic cannula. The perfusate was Krebs-Henseleit buffer containing (in mM) NaCl (120), KCl (4.7), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $CaCl_2$ (1.25), $NaHCO_3$ (25), and glucose (5). The buffer was continuously aerated with humidified 95% O_2/5% CO_2 and was maintained at 37 °C.

For assessment of contractile function, a rubber balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a Statham P23d pressure transducer that was outside the magnet at the same height as the heart. The balloon was inflated to give an end diastolic pressure of 5–15 cm water.

After 20 min of control noncirculating perfusion the perfusate was changed to a Krebs-Henseleit solution in which 2.5 mM CsCl replaced an equivalent concentration of KCl.

NMR Experiments. Cesium-133 NMR spectra of the erythrocyte preparation were taken at 34 °C in the Fourier transform mode by using a General Electric GN-500 NMR spectrometer operated at 65.6 MHz (B_0 = 11.75 T). The spectra were accumulated in 2K (real) data sets for approximately 17 min (500 scans) at regular intervals, with quadrature detection, a spectral width of ±1000 Hz, and a 2-s delay between excitation pulses. The flip angle of the excitation pulse was set at 45° (20 μs). Spectra of $^{133}Cs^+$ from the perfused rat heart were taken at 37 °C on a Nicolet NT-360 wide bore spectrometer operated at 47.4 MHz (B_0 = 8.5 T). The heart was suspended in a chamber that fit into the 20-mm broad-band probe unit of this spectrometer. Spectra were accumulated in 2K (real) blocks over 13-min intervals (256 scans) for a total period of 3 h. The spectral width was ±3000 Hz and the flip angle was 60° (40 μs); the delay between pulses was 0.84 s.

Spin–lattice relaxation times were measured by using an inversion–recovery pulse sequence: predelay–180°–τ–90°–acquire–T. T_1 values were determined by fitting the integrated intensities of the partially recovered signals with a three-parameter curve-fitting routine provided with the GE software. For the T_1 measurements of the extracellular Cs^+ resonance, four transients were acquired per delay, τ, with a predelay of 100 s between each acquisition. For the intracellular component, 16 transients were acquired for each τ value and the delay between acquisitions was 20 s.

Measurements of the T_2 value for the intracellular Cs^+ resonance in the erythrocyte preparation were made by determining the decay rates of spin echos generated via a simple Hahn echo sequence, 90–τ–180–τ–acquire, and via a Carr–Purcell–Meiboom–Gill sequence, $90°_x$–$(\tau$–$180°_y$–$\tau)_{2n}$–acquire. The echo amplitude was taken as the integrated intensity of the signal generated by Fourier transformation of the half-echo acquired with the receiver gated on after the last refocusing delay. The 180° refocusing pulse in both sequences was, in fact, a composite pulse, $90°_x180°_y90°_x$, and the phases of the radio-frequency pulses and receiver were cycled according to

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

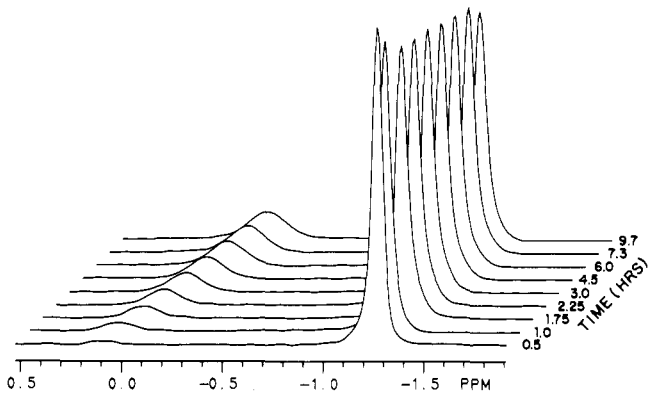

FIGURE 1: Cesium-133 NMR spectra (65.6 MHz) of human erythrocytes suspended in a buffer containing 140 mM NaCl and 10 mM CsCl. Spectra were accumulated for 20-min intervals ending at the times indicated in the figure. The free induction decays were multiplied by a 10-Hz Gaussian filter function before Fourier transformation. The origin of the chemical shift scale is arbitrary.

the EXORCYCLE scheme to correct for any imperfections in the setting of the pulse widths (Levitt & Freeman, 1981).

RESULTS

A series of $^{133}Cs^+$ NMR spectra obtained at 34 °C from human erythrocytes suspended at a hematocrit of 38 ± 1% in a modified Hank's buffer containing no K^+ and 10 mM Cs^+ are shown in Figure 1. As is apparent from the figure, the sharp, initially observed resonance at −1.25 ppm is augmented by a second, broader resonance 1.4 ppm downfield, which increases in intensity during the course of the experiment. On the basis of the observed time dependence of the spectra, the low-field resonance is assigned to intracellular Cs^+ and the upfield line to extracellular Cs^+.

The relative intensities of the intra- and extracellular components of the Cs^+ spectra shown in Figure 1 are somewhat distorted owing to the moderately fast pulse repetition rate used here and the approximately 3-fold difference in spin–lattice relaxation times for the two components (T_1^{intra} = 4.5 s; T_1^{extra} = 13.6 s). When these "T_1 distortions" are corrected for by using a formula derived by Ernst and Anderson (1966) to describe the steady-state signal behavior in a periodically repeated Fourier transform NMR experiment (Ernst & Anderson, 1966), one finds that the total intensity of the spectra is conserved and the concentration of intracellular Cs^+ ions may be determined as a function of the incubation time in the Cs^+ buffer. The time dependence of Cs^+ uptake by human erythrocytes suspended in buffers containing 10 mM Cs^+ or 10 mM Cs^+ plus 10^{-4} M ouabain is shown in Figure 2. Clearly, 10^{-4} M ouabain slows the rate of Cs^+ uptake. From the initial slopes of the curves in Figure 2, we determine rates of 0.6×10^{-3} (moles of Cs^+ per liter of cells per hour) and 0.3×10^{-3} (moles of Cs^+ per liter of cells per hour) for normal and ouabain-treated cells, respectively. The rate of Cs^+ uptake in the untreated cells is approximately 0.3 times the rate for K^+ uptake (Love & Burch, 1953; Garrahan & Glynn, 1967). The 2-fold reduction of the Cs^+ uptake rate in 10^{-4} M ouabain is comparable in magnitude to the ouabain-inhibited uptake of K^+ or Rb^+ (Garrahan & Glynn, 1967).

Similar NMR studies were also carried out on a perfused rat heart. The spectra, obtained in 13-min intervals over a period of 1.5 h, are shown in Figure 3. The perfusate was a Krebs-Henseleit buffer modified to contain 2.5 mM CsCl and 2.5 mM KCl. As in the red blood cell preparation, a broad Cs^+ resonance, shifted 1.1 ppm downfield from the sharper signal of the perfusate, begins to grow in intensity and can be

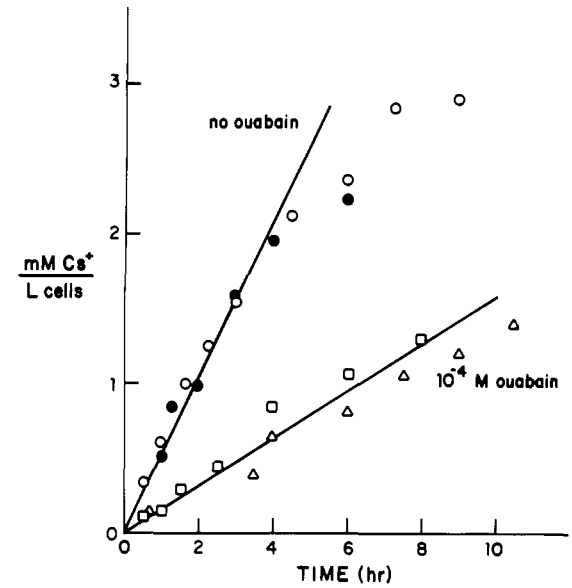

FIGURE 2: Concentration of intracellular Cs^+ in suspended human erythrocytes as a function of incubation time in buffer containing 10 mM CsCl. The different symbols refer to separate experiments. Concentrations were calculated from the equation shown below. I's

$$[Cs]_{cell} = (I_{cell}/I_{total})\frac{\text{extracellular volume}}{\text{cell volume}}[Cs^+]_0$$

are the integrated intensities of the appropriate Cs^+ resonances (corrected for relaxation), $[Cs^+]_0$ is the initial Cs^+ concentration of the buffer, and the volumes are derived from the hematocrit.

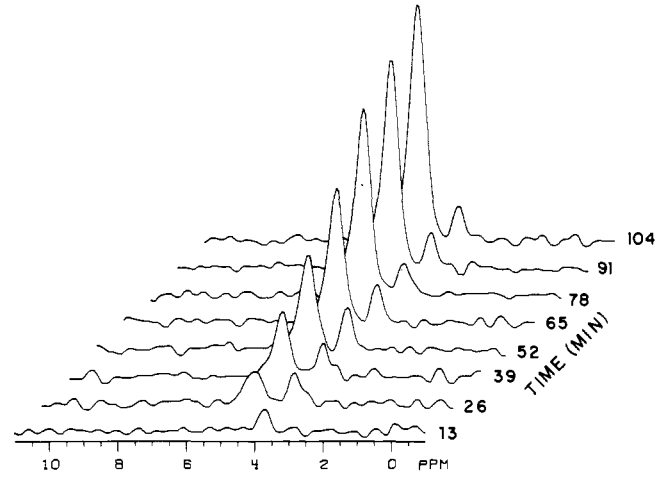

FIGURE 3: Cesium-133 NMR spectra (47.4 MHz) of a beating rat heart perfused with a buffer containing 2.5 mM CsCl. Spectra were collected continuously and stored in separate data blocks every 13 min. A Gaussian filter function of 20 Hz was applied to the data before Fourier transformation. As in Figure 1 the chemical shift scale is arbitrary. The homogeneity of the applied B_0 magnetic field was optimized by shiming on the ^1H NMR signal from water prior to CsCl perfusion.

assigned to Cs^+ ions taken up by the myocardium from the perfusate. Immediately upon perfusion with buffer containing 2.5 mM Cs, pressure development decreased by ~75%; over the next 30–40 min pressure gradually recovered to ~60–70% of its control (pre-Cs) level. A T_1 inversion–recovery experiment, carried out after ca. 2 h of Cs^+ ion accumulation, indicates that the intracellular signal component recovers with a single exponential time constant, T_1, of 2.5 s. The T_1 value measured for the buffer alone is 12 s.

After correction of the integrated intensities for T_1 distortions and the relative fluid volumes of the myocardium and the perfusate [0.6 and 0.4, respectively (Gonzales et al., 1980)], the ratio of the moles of intracellular to extracellular Cs^+ can

be determined as a function of the perfusion time. Over a time course of 3 h there is a steady accumulation of intracellular Cs^+ ions at a rate of 4 (±0.5) × 10^{-3} (moles per liter of cell water per hour) and no indication of an approach to a steady state.

Discussion

The results of these Cs NMR experiments demonstrate several properties of Cs ions in biological samples: (1) intracellular and extracellular Cs^+ ions have different chemical shifts that are readily resolved without the use of shift reagents; (2) spin–lattice relaxation times for intracellular cesium ions are significantly shorter than the values of extracellular ions; (3) in red blood cells, Cs^+ ions are taken up at a rate approximately one-third that of K^+ ions; (4) the rate of Cs^+ ion uptake into red blood cells is decreased in the presence of the cardiac glycoside, ouabain.

The most striking and novel feature of these results is that intracellular and extracellular Cs^+ resonances can be resolved without the aid of shift reagents. At present we can only speculate as to the origin of these shifts, which, relative to the extracellular buffer, are approximately 1.0 and 1.5 ppm downfield for Cs^+ in rat heart and erythrocytes, respectively.

It is well-known that the chemical shift of the Cs^+ is exquisitely sensitive to its environment and depends on both the concentration and the nature of the counterions present, as well as on the solvent composition and temperature (Halliday et al., 1969). In aqueous solutions, for example, the Cs^+ chemical shift shows a nearly linear, lowfield shift with respect to the anion concentration with a slope dependence such that $[I^-] > [Br^-] > [Cl^-]$ (Halliday et al., 1969). Moreover, the anion-induced shifts are additive and essentially independent of other cations present in the solution. With the buffer solutions used here, the extracellular Cs^+ shift (measured relative to the shift at infinite dilution) is determined principally by Cl^- ions, present at 1.5 × 10^{-1} M.

Since the intracellular Cs^+ resonance is shifted downfield with respect to its shift in the buffer solutions, the intracellular Cl^- concentration cannot be the only factor influencing the intracellular Cs^+ shift. If it were, one would expect an upfield shift since $[Cl^-]_{in} < [Cl^-]_{out}$ (Jay & Burton, 1969). One contribution, which would add to the effect of $[Cl^-]_{in}$ to produce a low-field shift relative to the extracellular buffer, could arise from negatively charged phosphate groups, present intracellularly as inorganic phosphates $H_2PO_4^-$ and HPO_4^{2-} as well as in the form of numerous organic phosphate derivatives. In fact, measurements of the Cs^+ chemical shift as a function of the total phosphate ion concentration at pH 7 show a slope with respect to concentration that is approximately twice that for $[Cl^-]$. Since intracellular phosphates are significant, if not predominant, carriers of negative charge in most cells, it is reasonable to propose that the low-field shift of the intracellular Cs^+ resonance reflects the combined influence of both intracellular Cl^- and negatively charged phosphate groups. We note, however, that given the extreme sensitivity of the Cs^+ chemical shift to solvent conditions, deviations of cytosolic H_2O from nonideality could also influence the Cs^+ shift quite independently of the influence exerted by the concentrations and types of intracellular counterions.

The ability to resolve and observe subtle differences in the chemical environment of $^{133}Cs^+$ is, in part, a consequence of the small quadrupole moment and attendant T_1^{-1} and T_2^{-1} relaxation rates of ^{133}Cs. Compared to ^{23}Na, ^{39}K, and ^{85}Rb, ^{133}Cs relaxation rates in solution are approximately 200 times smaller (Lindman & Forsen, 1978), thus providing a distinct advantage over the other alkali metal ions in NMR studies of biological systems: namely, the increased potential for complete "visibility".

Owing to transient interactions with macromolecules, quadrupolar nuclei with spin $I > 1$ show complicated relaxation behavior (Hubbard, 1970; Edzes & Berendsen, 1975). In particular, there is an enhanced and progressive broadening of the line widths associated with transitions, $|\Delta M_I| = 1$, involving spin states with quantum numbers, $|M_I| > {}^1/_2$. For ^{23}Na and ^{39}K ($I = {}^3/_2$), the $|{}^1/_2| \leftrightarrow |{}^3/_2|$ transitions are often broadened to the extent that these transitions cannot be observed with conventional NMR spectrometers because of finite pulse widths and receiver dead times (Lerner & Torchia, 1986). For these nuclei only the $-{}^1/_2 \leftrightarrow {}^1/_2$ transition, representing 40% of the total transverse magnetization, may be "visible". Although ^{133}Cs, with spin $I = {}^7/_2$, has even more complex relaxation behavior than the spin $I = {}^3/_2$ nuclei (Bull et al., 1979), the extent of broadening of the ^{133}Cs spin transitions is limited by the extremely weak coupling of its spin states to the lattice and is therefore more likely to be within the accessible spectral bandwidth of the spectrometer.

In erythrocytes, we conclude that all the intracellular Cs^+ is observable for two reasons. First, the total intensity (intracellular plus extracellular) of the Cs^+ resonance, corrected for T_1 effects, is conserved throughout the course of the loading experiment. Second, measurements of the spin–spin relaxation time T_2, using a Hahn spin-echo sequence, show a single exponential decay of the intracellular Cs^+ intensity with a time constant T_2 of 0.33 (±0.01) s. This result also reveals that the intracellular line width ($\Delta\nu_{1/2} \approx 10$ Hz; $T_2^* = 0.033$ s) is dominated by inhomogeneous broadening, due either to slow exchange of the Cs^+ ions among magnetically nonequivalent sites or to a distribution of the Cs^+ ions over a population of cells with differing intracellular chemical shifts. Since T_2 values measured via the Carr–Purcell–Meiboom–Gill sequence are slightly longer and show a dependence on the echo refocusing rate [$T_2^{CPMG} = 0.48$ (±0.04) s and $2\tau = 16$ ms; $T_2^{CPMG} = 0.61$ (±0.02) s and $2\tau = 2$ ms], it would appear that some type of slow exchange process within the cell is causing the broadening here (Bloom et al., 1965). The total visibility of intracellular Cs^+ in erythrocytes is not exceptional and is consistent with the fact that NMR measurements of intracellular ^7Li, ^{23}Na, and ^{39}K yield accurate and reliable values for the concentrations of these ions.

In the perfused rat heart, the question of the visibility of the intracellular Cs^+ is more problematic for, unlike the erythrocyte loading experiment, we do not have a closed system with respect to total Cs^+ concentration and thus no "internal" intensity standard. The relaxation properties of intracellular Cs^+ in the heart are not grossly different from those of Cs^+ in erythrocytes. The spin–lattice relaxation times for Cs^+ in the heart is 2.5 s [T_1(perfusate) = 12 s], approximately 1.5 times shorter than for Cs^+ in erythrocytes. In view of the fact that quadrupolar contributions to the relaxation of Cs^+ are quite weak compared to the relaxation of ^{23}Na or ^{39}K, as well as the apparently exponential recovery of the magnetization in the T_1 study, our data suggest that one can indeed observe most of the intracellular Cs^+ in the heart. Clearly, further investigations are required to answer this question definitively.

In our experiments Cs^+ was loaded into cells with electrochemical potentials that were lower inside than outside. The fact that ouabain inhibited the inward Cs^+ flux indicates, however, that some component of the Cs^+ flux is carried by an active transport system such that the Cs^+ ions substitute for or compete with K^+ ions in the translocation process. Other studies with red blood cells indicate that Cs^+ can substitute

for K^+ in activating the Na/K-dependent ATPase and that the activation is accompanied by Cs^+ translocation (Whittam & Ager, 1969). In yeast, Cs^+ will inhibit K^+ transport, although the type of inhibition depends on the Cs^+ concentration (Armstrong & Rothstein, 1967). At high concentration, the inhibition of K^+ transport is competitive and the Cs^+ ion itself is transported. Clearly Cs^+ is not an adequate substitute for K^+ in terms of biological function. Nonetheless, Cs^+ does appear to sufficiently mimic properties of K^+ ions that it can be employed as a useful NMR probe or tracer to study general properties of K^+ transport and distribution in vivo.

The observation that a low concentration of Cs^+ in the perfusate does not seriously compromise the mechanical and electrical properties of the rat heart, coupled with the sensitivity of the Cs^+ chemical shift to its immediate environment, suggests that Cs^+ NMR could be used to report on changes in myocardial tissue induced by local and global ischemia as well as other types of cell injury. Moreover, the entire time course of Cs^+ uptake, tissue distribution, and excretion could be studied noninvasively in a single organism by Cs magnetic resonance imaging methods.

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