# Subcompartmental Cesium Diffusion in Healthy and Globally Ischemic Rat Brain

J. A. Goodman[1], J. J. Ackerman[1,2], J. J. Neil[3,4]

[1]Chemistry, Washington University in St. Louis, St. Louis, Missouri, United States, [2]Radiology, Washington University in St. Louis, St. Louis, Missouri, United States, [3]Pediatrics, Washington University in St. Louis, St. Louis, Missouri, United States, [4]Neurology, Washington University in St. Louis, St. Louis, Missouri, United States

**Introduction:** The ability to localize the MR signal from water or other ions to the intra or extracellular space in intact tissue is an important step in understanding the compartment specific changes that take place as a result of cell injury. Spectroscopic resolution of these compartments will allow investigators to probe the chemical and kinetic environments of those spaces directly, and thus observe how they change following injury. Inferring the compartment-specific kinetic environment of water is of special importance for understanding the biophysical mechanisms that underlie contrast of injured central nervous system (CNS) tissue in diffusion-weighted MRI. $^{133}$Cs, the ~100% naturally abundant isotope of cesium, is an MR-active physiologic analog of potassium and, in its cationic form, $Cs^+$, is actively taken up by cells and resides primarily in the intracellular space. Its chemical shift is sensitive to differences in the chemical composition of the compartment in which it resides (see references in (1)). $^{133}$Cs MR has been used to obtain compartmental, even subcompartmental, information on the kinetic environment of healthy and ischemic rat brain.

**Materials and Methods:** Ten 250 – 350 gram, male Sprague-Dawley rats were fed low potassium chow and water containing 20 mM $^{133}$CsCl and 20 mM KCl. Under this regimen, cesium can be observed by MR within several days. Each rat was kept on the diet for a minimum of one week prior to being scanned. Experiments were performed on an 11.74 T horizontal bore scanner (Magnex Scientific/Varian Instruments). Animals were loaded into an MR-compliant head restraint equipped with a nose cone, ear bars, and a bite bar. A 4-cm proton surface coil (500 MHz) was placed on the side of the animals head and was used for scout imaging, voxel planning, and localized shimming. A 1.0 x 1.4-cm, two-turn ellipsoidal transmit/receive $^{133}$Cs surface coil (65 MHz) was placed on the rat's head, directly above the brain. $^{133}$Cs diffusion, $T_1$, and $T_2$ measurements were made with a LASER sequence modified to provide only one dimension of localization (a slice parallel to the plane of the RF coil), selecting a cylindrical slab in the brain. $^{133}$Cs ADCs were estimated using 6 logarithmically spaced *b* values from 0.001 to 2.951 ms/μm$^2$ (tr=3.8 s, te=44 ms, δ=10 ms, Δ=22 ms). Global ischemia was induced within the magnet *via* a 2M KCl bolus delivered through a tail vein catheter, and the diffusion measurement was performed again, using six b-values from approximately 0.001 to 5 ms/μm$^2$ (tr=3.8 s, te=48 ms, δ=12 ms, Δ=24 ms). Water diffusion measurements were also performed before each experiment using a LASER sequence equipped with diffusion weighting pulses. Following death, brains were kept warm by blowing warm air over the animal's head.

**Results** **Relaxation:** Three resonances are evident in the spectrum shown in figure 1. The signal to noise ratios acquired in the $^{133}$Cs $T_1$ and $T_2$ measurements only permitted meaningful analyses of the two peaks at 0.0 ppm and -0.7 ppm. The results are shown in table 1. The relative signal fraction of each peak is also given in table 1. These fractions are corrected for tr and te using the measured $T_1$ and $T_2$ relaxation time constants (with the average $T_1$ and $T_2$ used to estimate the contribution of the peak at -2.9 ppm). **Diffusion:** The measured ADCs in healthy and ischemic brain are presented in table 2. The diffusion signal attenuation data for all peaks were modeled using a single exponential function. Only peaks at 0 and -0.7 ppm were analyzed because of signal to noise ratio limitations. In healthy brain tissue, these two resonances have roughly equivalent ADCs: 1.0 and 0.9 μm$^2$/ms, respectively. Upon death, the resonance at -0.7 ppm drops approximately 75 %, to 0.24 μm$^2$/ms, while the resonance at 0.0 ppm remains unchanged. Diffusion spectra from one of the sacrificed animals are shown in figure 2. Upon global ischemia, water diffusion decreased from 1.02 ± 0.07 μm$^2$/ms to 0.52 ± 0.05 μm$^2$/ms.

**Discussion** **Peak assignment:** There are three lines of evidence suggesting that the resonance at -2.9 ppm represents Cs in the extracellular space. The first is related to resonance amplitudes. Cesium concentration, when introduced in this manner, has been estimated to be nine times higher in the intracellular space than in the extracellular space (2). Assuming that the intracellular space occupies 80% of total brain tissue, and assuming similar $T_1$s and $T_2$s in both spaces, the extracellular resonance would be expected to be 2.2 % of the total signal. The amplitude of the resonance at -2.9 ppm (modeled in green) represents 2.4 % of the total signal when corrected as described above. Second, we have observed that the resonance at -2.9 ppm is shifted to higher frequency following intracerebroventricular infusion of $Co(CN)_6$ into the extracellular space (3), while the frequencies of the other two resonances are unaffected (data not shown). Finally, this peak assignment is supported by data from perfused hepatocyte studies (4). It has been suggested that the two other large peaks represent $^{133}Cs^+$ in the cytosol (0.0 ppm) and subcellular organelles (-0.7 ppm), such as the mitochondria (4). **Diffusivity:** To the extent that that cesium is solvated, it should reflect the motion of water in the compartment in which it resides. In the case of the two intracellular $^{133}$Cs resonances evaluated in this study, the ADC value of the cytosolic resonance showed no change following injury, whereas the ADC of the resonance attributed to Cs in organelles such as mitochondria shows a very large decrease. This suggests that the decrease in intracellular water ADC associated with cell injury is not uniform across different subcellular compartments and is due mainly to a decrease in the ADC of water in organelles. An alternate explanation is that the two large resonances represent two different cell types with different $^{133}$Cs chemical shifts.

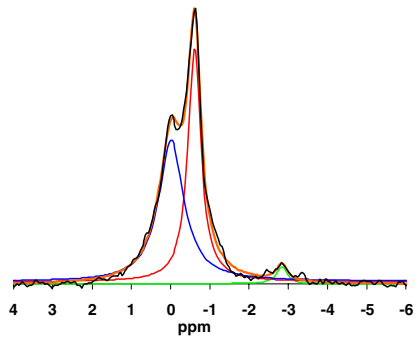


**Figure 1.** A $^{133}$Cs spectrum of rat brain, shown with 10 Hz line broadening. tr=3.4 s, te=28 ms, 512 transients, total acquisition time=21 minutes. The black line is the filtered spectrum, the red, blue, and green lines represent the three modeled peaks. The orange line is the sum of the modeled peaks.

| peak, ppm | corrected fractions | $T_1$, s | $T_2$, ms |
|---|---|---|---|
| -2.9 | 0.025 ± 0.010 | | |
| -0.7 | 0.32 ± 0.15 | 3.0 ± 0.3 | 35 ± 7 |
| 0.0 | 0.65 ± 0.14 | 3.5 ± 0.5 | 26 ± 3 |

**Table 1**. Column one contains estimated fractional contributions of each resonance to the total signal, corrected for tr and te. Average $T_1$ and $T_2$ time constants are given for five animals. All ± values represent inter-animal standard deviations.

**This research was funded by NIH grants NS35912 and R24-CA83060.**

| | peak, ppm | ADC, μm$^2$/ms | average uncertainty | standard deviation | relative fraction |
|---|---|---|---|---|---|
| healthy | -0.7 | 0.9 | 0.2 | 0.4 | 0.35 |
| n=10 | 0 | 1.0 | 0.1 | 0.2 | 0.65 |
| ischemic | -0.7 | 0.24 | 0.04 | 0.1 | |
| n=8 | 0 | 1.0 | 0.3 | 0.2 | |

**Table 2**. ADCs for the two primary intracellular $^{133}Cs^+$ resonances. Columns three and four represent the average estimated uncertainty for a single measurement and the inter-animal standard deviation, respectively. The relative fractions do not change appreciably from healthy to ischemic brain.

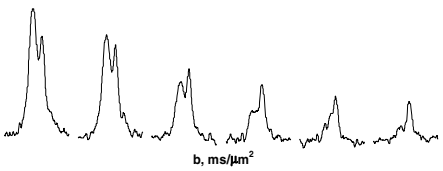


**Figure 2**. A representative array of diffusion attenuated $^{133}Cs^+$ resonances acquired at *b* values of 0.001, 0.208, 0.816, 1.824, 3.231, and 5.039 ms/μm$^2$. The two ADCs are clearly evident. This measurement took ~ 24 minutes.

**References:**

1. Goodman J, Neil JJ, Ackerman JJH. *NMR Biomed.* 2005; 18: 125-134.
2. Li Y, Neil J, Ackerman JJH. *NMR Biomed.* 1995; **8**: 183-189.
3. Askenasy N, Navon G. *NMR Biomed.* 2005; **18**: 104-110.
4. Wellard RM, Adam WR. *Magn. Reson. Med.* 2002; **48**: 810-818.