MEASUREMENT OF TISSUE POTASSIUM IN VIVO USING ^{39}K NUCLEAR MAGNETIC RESONANCE

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ABSTRACT ^{39}K nuclear magnetic resonance (NMR) spectra were readily obtained, in vivo, from rat muscle, kidney, and brain in 5–10 min with signal-to-noise ratios of ~20:1. Quantitation of the K^+ signal was achieved by reference to an external standard of KCl/dysprosium nitrate as well as by reference to the proton signal from tissue water. In vitro NMR studies of isolated tissue showed a K^+ visibility (NMR K^+/total tissue K^+) of 96%, 62 ± 8%, 47 ± 1.9%, 45 ± 3.5%, and 43 ± 2.5% for blood, brain, muscle, kidney, and liver, respectively. Absolute tissue K^+ was determined by flame photometry of acid-digested tissue. Changes in tissue K^+ status by chronic K^+ depletion or acute K^+ loading produced changes of ^{39}K NMR signal intensity that were equal to changes of absolute tissue K^+. Acidosis, alkalosis, mannitol, or RbCl infusion did not significantly change the NMR K^+ signal. These results indicate that the changes in K^+ detected by NMR were specifically and accurately detected. To investigate the factors that affect the ^{39}K NMR signal, the effects of liver homogenate on ^{39}K NMR signal intensity were studied. Addition of homogenate produced a 60% loss of signal intensity, suggesting that a large portion of cell K^+ may be only 40% visible. Addition of RbCl to undiluted homogenate increased the NMR K^+ signal by 11 ± 2 μmol/g. Addition of H_2O or NaCl had no effect, suggesting that Rb^+ was replacing K^+ in sites of low (< 40%) NMR visibility. These results demonstrate that ^{39}K NMR experiments can be performed using intact organs. To explain the lack of detectable K^+ and changes in K^+ NMR visibility, a three compartment model is proposed.

INTRODUCTION

Potassium (K^+) is the major ionic constituent of cells. Changes of intracellular K^+ are associated with a wide range of physiological effects. For example, K^+ depletion in whole animals is associated with impaired carbohydrate and protein metabolism, neuromuscular and cardiac toxicity, and abnormalities of renal function (see reference 1 for a review). Because 98% of whole body potassium is intracellular, measurement of plasma K^+ concentrations are often a very insensitive index of tissue K^+ stores. Previous methods for measuring tissue K^+ concentrations have involved biopsies, whole body counting techniques of ^{40}K, or tracer studies with short half life $^{42}K^+$ (see reference 2 for a review). In isolated cell systems microelectrodes have been used to measure intracellular K^+ activities, but questions concerning the specificity and calibration of these electrodes remain (2–4). A rapid, non-invasive method to measure tissue K^+ would be useful for clinical diagnosis as well as for investigation of the role of K^+ in cellular metabolism.

^{39}K is the naturally abundant potassium isotope (93%). It possesses a nuclear spin 3/2 and thus may be detected by nuclear magnetic resonance (NMR) spectroscopy. Previous investigators have detected ^{39}K NMR signals from isolated cells (5, 6) and isolated perfused tissues (7). The low sensitivity (0.05% of ^1H) and the potential complications due to the interaction between the quadrupole moment of the K^+ ion and local electric field gradients in the cell (8) have discouraged attempts to measure K^+ using NMR in tissues in situ.

The goals of the present experiments were (*a*) to determine the feasibility of detecting ^{39}K NMR signals from rat tissues in situ, (*b*) to determine whether or not the ^{39}K NMR signal detected represents the total K^+ content of the tissue, and (*c*) to see how physiologic maneuvers that change intracellular K^+ levels alter the ^{39}K NMR signal intensity.

METHODS

Animal Methods

Female Sprague Dawley rats (Simonsen, Gilroy, CA) weighing 180–220 g were fed standard rat chow or a low K^+ diet (Teklad, Madison, WI) for 5–21 d before the study. Chronic metabolic acidosis and chronic metabolic alkalosis were produced by provision of either 0.28 M NH_4Cl or 0.28 M $NaHCO_3$ as sole drinking fluid for 5 d. Acute electrolyte changes were produced by intraperitoneal injection of 5 ml/100 g body weight of 0.2 M

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KCl, RbCl, $NaHCO_3$, NH_4Cl, or 12.5 g/dl Mannitol. NMR spectra were obtained for 1 h post-injection. Tissue K^+ was measured by flame photometry (Instrumentation Laboratory, Inc., Lexington, MA), using nitric acid digests of appropriate tissue. Liver was homogenized, without additional medium, with a polytron (model PCU2; Polytron Instruments), setting 6, for 6 s. The pellet was separated from the supernatuant after centrifugation at 50,000 *g* for 2 h.

NMR Methods

^{39}K is a quadrupole nucleus with a large quadrupole coupling constant, therefore NMR signals from immobilized K^+ ions will be extremely broad. All experiments were performed under conditions that could detect only relatively narrow (<2 kHz) signals. Throughout this paper the expression "NMR visible" means that under these high resolution conditions any broad ^{39}K signal could not be detected.

In vitro NMR studies were performed on various tissues within 1 h of death produced by stunning and cervical dislocation. 2–3 g of tissue were examined using a 7-turn solenoidal coil, made of 16-gauge wire, 2.2 cm long, and 1.7 cm internal diameter. In vivo NMR studies were performed on rats anesthetized with Ketalar and Rompun, as described previously (9), utilizing a probe bed maintained at 38°C with circulating water. For muscle K^+, a 6-turn oval coil made of 16-gauge wire 1.5 cm long, 1.5 cm minimum diameter, and 2.5 cm maximum diameter was placed around the rat's thigh. For kidney K^+, a 4-turn coil was surgically implanted around the rat kidney, with a Gortex shield (Gortex Corp., Sunnyvale, CA) between the coil and adjacent muscle (9). K^+ spectra were obtained from brain and adjacent tissues of the skull with a 6-turn solenoidal coil surrounding the rat's skull. To facilitate quantitation in both in vitro and in vivo studies, an external standard of 200 μl of 3 M KCl with 200 mM dysprosium nitrate was attached to the exterior of the coils. This was used to account for alterations of coil sensitivity. The coil was calibrated with phantoms containing 20–100 μmol KCl placed inside the coil. The coils were tuned to 11.05 MHz, the frequency for ^{39}K in the 5.8-T, 9-cm-diam, horizontal bore superconducting magnet (Nalorac Instrument Corp., Martinez, CA). ^1H signals from tissue water were used to shim before obtaining the ^{39}K spectra. Spectra were obtained with pulses that produced a maximum signal, characteristically 30–100 μs for ^1H, and 40–100 μs for ^{39}K. Delay between pulses for ^{39}K NMR was 0.3 s. Increasing the delay time caused no change in signal intensity indicating that the K^+ was able to fully relax during this interval, consistent with the short T_1 usually associated with quadrupolar nuclei (8). Signal-to-noise ratios for ^{39}K spectra were >20 with 500–1,000 pulses, requiring 2–5 min of acquisition time.

The potassium content of tissues in vitro was quantitated using the slope of the relationship between ^{39}K (in millimolars) and signal intensity obtained from phantom measurements. The problem of standardization was more difficult in vivo because the volume of tissue detected by the coil could not be accurately quantitated. Approximate quantitation was achieved using the method of Thulborn and Ackerman (10). Precise 90° pulses were difficult to obtain due to the large size of the coils. Therefore, the ratio of the maximum ^{39}K to maximum ^1H signal in the tissue was compared with that of the KCl standard in a tube extending well beyond the coil. For all in vivo studies, changes in the K^+ signal were related to the initial control values, using the external reference as an integration standard. NMR K^+ visibility is defined as the measured K^+ by NMR as a percent of the total potassium determined by flame photometry of a nitric acid digest. The results were adjusted assuming that the $^{39}K^+$ isotope was 93% of total K^+ in all samples. The results are expressed as mean ± standard error of the mean. Significance between means was determined using the paired or unpaired Student's *t* test.

RESULTS

^{39}K NMR of Tissue In Vivo

^{39}K NMR spectra were readily obtained in vivo from muscle, kidney, and brain with a signal-to-noise ratio exceeding 20:1 with 1,000 accumulations in 5–6 min (Fig. 1). The line widths of spectra obtained in vivo were ~100 Hz. The resonance of the external standard of KCl was shifted 400 Hz downfield from the tissue signal due to dysprosium nitrate (Fig. 2). The spectra were stable for up to 8 h. Repeated measurements on one sample produced a coefficient of variation of 13%.

To determine the effects of tissue viability on the ^{39}K NMR spectrum, rats were killed with an overdose of anesthesia, with continuous recording of the ^{39}K signal from thigh muscle. Fig. 3 demonstrates no change in signal intensity for the first hour after death. However, 3–5 h after death, the ^{39}K NMR signal significantly increased, reaching a maximum increase of ~30% above baseline in 9 h ($P < 0.01$). The stability of the tissue K^+ signal for 2 h after death allowed in vitro studies to be performed on excised tissue. Because the total tissue K^+ does not change after death, the later rise in NMR signal represents a change in NMR visibility (discussed below).

^{39}K NMR of Tissue In Vitro

To determine the proportion of tissue K observed by ^{39}K NMR, spectra of various isolated tissues were obtained and the results compared with total K content measured from tissue extracts. Table I shows that the NMR visibility of K varies over a wide range, depending on the tissue type. K^+ visibility in liver and kidney was 43–45%. Muscle

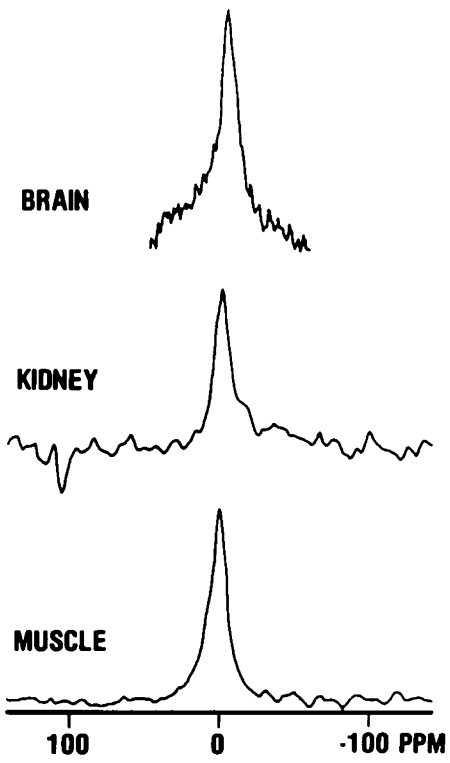

FIGURE 1 NMR K^+ spectrum of in vivo rat thigh muscle, kidney, and skull (brain). The spectra were obtained with 1,000 accumulations using a 90° pulse and 0.3-s delay between acquisitions. 30 Hz line broadening was applied to the FID before Fourier transformation.

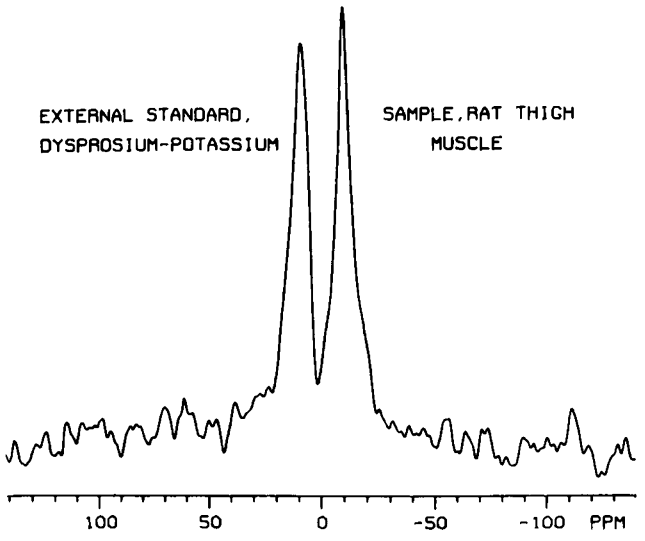

FIGURE 2 NMR K^+ spectrum of in vivo rat thigh muscle showing the KCl/dysprosium nitrate external standard shifted downfield from the tissue signal. The external standard provided a constant for integration and quantitation of the sample.

visibility was 49 ± 2%; brain, 62 ± 1%; and blood, 96%. These findings demonstrate that the ^{39}K NMR signal detected under these conditions cannot be used as an absolute measure of K^+ concentration of tissues in vivo and in vitro. Furthermore, they indicate that the factors that alter K^+ visibility are variable from tissue to tissue, suggesting the possibility that the visibility of K^+ in a specific tissue may also be variable.

Comparison of ^{39}K NMR with Absolute Tissue K^+ Content

Experiments were performed to determine if ^{39}K NMR was able to detect changes in tissue K^+ content (measured by flame photometry) produced by alterations of whole body K^+ homeostasis. Fig. 4 demonstrates that alterations of K^+ homeostasis had a major effect on the ^{39}K NMR signal intensity. Dietary-induced muscle K^+ depletion produced a proportionate decrease of the ^{39}K NMR signal. With acute infusion of KCl, the muscle ^{39}K NMR signal rose by 25^+ ($P < 0.01$) whereas the total muscle K^+ rose by only 12% (Figs. 4 and 5, Table II). Linear regression analysis of the experiments illustrated in Fig. 4 gave a line of best fit described by NMR K^+ = 0.96 (muscle K^+) − 45.6 M/g ($n = 27, r = 0.92$). The slope (0.96) suggests that K^+ added to or removed from muscle was totally detected by NMR. This was true despite the fact that only a fraction of total muscle K^+ was detected by NMR.

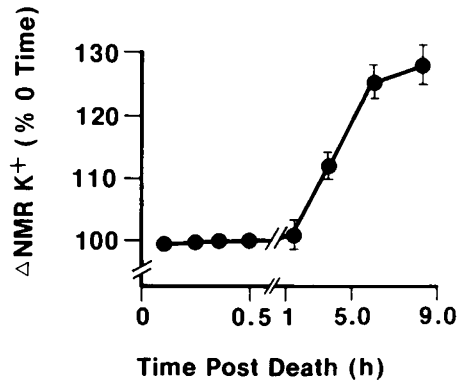

FIGURE 3 The effect of death on NMR visible K^+ in rat thigh muscle. Basal measurements were made and then the rats were killed by an overdose of anesthetic. The post-mortem measurements were made without removing the animals from the magnet.

TABLE I
NMR K^+ VISIBILITY IN DIFFERENT TISSUES

Tissue	NMR K^+	Tissue K^+	NMR visibility
	μmol/g	*μmol/g*	%
Blood	43	45	96
Brain	59 ± 1.0	95 ± 1.0	62 ± 8.0
Muscle	47 ± 1.5	99 ± 1.1	47 ± 1.9
Kidney	28 ± 2.9	62 ± 0.5	45 ± 3.5
Liver	34 ± 2.9	80 ± 1.1	43 ± 2.5

K^+ depletion produced a 17-μmol/g change, determined by NMR and a 20-μmol/g change determined by flame photometry. K^+ loading produced an increase of 15 μmol/g measured by NMR and a 12-μmol/g increase measured by flame photometry. Similar results were found for the liver of K^+ depleted rate (Table II). The decrease in K detected by NMR (10 μmol/g) was equal to the fall determined by flame photometry (10 μmol/g). Although, the absolute changes in K^+ determined by the two techniques were similar, the initial values were quite different. Therefore, alterations in K^+ homeostasis changed the fraction of total K^+ that was NMR visible (Table II). This indicates that the values for percent visibility of K^+ (e.g., in Table I) are highly dependent on the physiological state of the animal.

To determine if changes associated with K^+ depletion could be measured by NMR in vivo, the K^+ signal was normalized to proton content by a modification of the method of Thulborn and Ackerman (see Methods). Using this method, muscle K^+ depletion of 20% (20 μmol/g) was

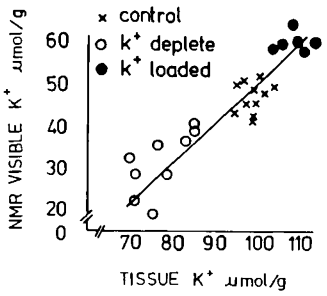

FIGURE 4 Comparison of ^{39}K NMR and absolute tissue content muscle K^+. The symbols represent: *crosses*, control; *open circles*, dietary K^+ depletion; *solid circles*, acute K loading. NMR K^+ clearly correlated with tissue K^+. (*Solid line*) The best fitted regression line with a slope of 0.96.

TABLE II
THE EFFECT OF CHANGE IN TISSUE K^+ ON NMR K^+ IN VIVO AND IN VITRO

Tissue	K Status	*n*	NMR K^+	ΔNMR K^+	Tissue K^+	ΔTissue K^+	Tissue K^+ NMR visible
			μmol/g	*μmol/g*	*μmol/g*	*μmol/g*	%
Muscle	Control	11	47 ± 1.5	—	99 ± 1.1	—	47 ± 1.9
	K^+ depletion (in vitro)	9	30 ± 3.7	−17 ± 3.7	79 ± 2.3	−20 ± 2.3	39 ± 3
	K^+ depletion* (in vivo)	7	28 ± 1.9	−19 ± 1.9	81 ± 2.2	−18 ± 2.2	34 ± 2.5
	K^+ load* (in vivo)	6	62 ± 4.8	15 ± 3.0	111 ± 3.7	12 ± 1.8	56 ± 3
Liver	Control	7	34 ± 2.9	—	80 ± 1.1	—	43 ± 2.5
	K^+ depletion (in vitro)	5	24 ± 3	−10 ± 3	70 ± 4.6	−10 ± 4.6	35 ± 1.2

*As muscle NMR K^+ does not change shortly after death so the control muscle NMR K^+ in vivo has been allocated the value obtained in vitro, 47 μmol/g.

associated with a 40% fall of the ^{39}K NMR signal. Assuming that NMR detects 49 μmol K^+/g in controls (Table I), the deficit in the K^+ deplete rats measured by NMR was 17 μmol/g. This is no different from the tissue K^+ deficit measured by flame photometry (Table II). These results are virtually identical to those obtained on excised tissue (Table II). These findings suggest that NMR may be used to measure disturbances of tissue K^+ in vivo.

Effects of Other Fluid and Electrolyte Changes on the ^{39}K NMR Signal

To determine if the effects of K^+ loading and depletion on ^{39}K NMR spectra were specific to changes in K^+ concentration and not to nonspecific effects (e.g., changes of osmotic or acid–base status), experiments were performed to study the effects of these variables.

NMR spectra were obtained from muscle excised from rats treated with NH_4Cl to produce acute and chronic acidosis, $NaHCO_3$ to produce acute and chronic alkalosis, and mannitol to produce loss of water from cells. Table III demonstrates that none of these maneuvers had a significant effect on the fraction of total K^+ determined by NMR in skeletal muscle; the percent visibility of K^+ was constant at 40–50%.

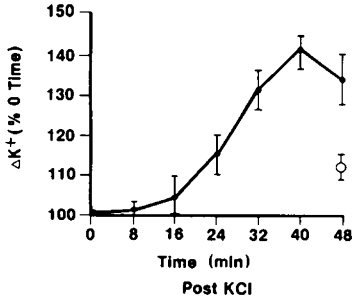

FIGURE 5 The effect of an acute infusion of 2.0 ml of a 0.5 M KCl solution on NMR visible K^+ (*solid circle*) and tissue K (*open circle*) in vivo.

In addition to the above experiments, RbCl, equimolar to the amount of KCl in the K^+ loading experiments, was infused, Rb^+ substitutes for K^+ in the Na-K ATPase and replaces K^+ in the intracellular space (11). A RbCl load might be expected to increase cellular Rb^+ and cause cell swelling similar to the K^+ load. RbCl had no effect on the K^+ signal (Table III).

These results indicate that changes in ^{39}K NMR spectra are due to changes in K^+ content and are not influenced by factors tha rt might change K^+ visibility, such as alterations of osmolality or acid–base status.

Factors that Alter ^{39}K NMR Visibility

Fig. 2 demonstrates an increase in signal intensity after death, indicating that ^{39}K NMR intensity may change

TABLE III
EFFECTS OF ELECTROLYTE AND ACID-BASE DISTURBANCES ON MUSCLE POTASSIUM

Treatment	*n*	ΔNMR K^+	ΔTissue K^+
		μmol/g	*μmol/g*
Chronic K depletion	9	−17 ± 3.2	−20 ± 1.1
Chronic NH_4Cl	5	− 3 ± 1.4	5 ± 1.1
Chronic $NaHCO_3$	5	− 1 ± 1.3	6 ± 1.3
Acute KCl	7	15 ± 3.0	12 ± 1.8
Acute RbCl	5	4 ± 3.0	4 ± 2.0
Acute NH_4Cl	2	− 2.4	1
Acute $NaHCO_3$	2	6.1	− 1
Mannitol	2	− 0.5	1

The results are expressed as the change from control values taken from Table I.

without a change in tissue K^+ content. To investigate this further, experiments with liver homogenates were performed. Fig. 6 shows that when liver homogenate was added to 150 mmol/liter of KCl, a progressive loss of ^{39}K NMR signal was produced, to ~40% of total K^+ content. This extent of signal quenching is similar to that seen with ^{23}Na NMR of liver homogenate (12). The concentration of liver homogenate at which maximum diminution of signal occurred with about a one-third dilution (liver homogenate to KCl). A more concentrated suspension of liver homogenate did not reduce the K^+ signal below 40%. This effect of liver homogenate on the ^{39}K NMR signal could not be reproduced by the addition of up to 25 g/100 ml albumin or by addition of the supernatant from the liver homogenate after centrifugation for 2 h at 50,000 *g*. To determine what portion of the K^+ was sequestered in compartments of <40% visibility, 200 μmol/g RbCl was added to undiluted liver homogenates. The addition of 200 μmol/g RbCl to undiluted liver homogenates produced an increase in ^{39}K signal of 11 ± 2 μmol/g ($P < 0.01$). This effect was not observed by dilution with equivalent amounts of water or NaCl, indicating that the increase in K^+ signal may be due to replacement of K^+, by Rb^+ from organelles, or compartments having a low NMR visibility. The 11 μmol/g increase in signal intensity produced by adding RbCl to a undiluted homogenate represents 14% of the total K^+ in liver (Table I). If it is assumed that 200 μmol/g Rb^+ replaces all sequestered K^+, then ~14% of liver K^+ may be residing in compartments of low NMR visibility.

DISCUSSION

The results of these experiments demonstrate that NMR is capable of detecting tissue K^+ in vivo. Furthermore, changes in tissue K^+ content, produced by K^+ loading or K^+ depletion, can be detected. In vitro, the percent of total tissue K^+ detected varies from tissue to tissue (Table I), from nearly 100% in red blood cells (confirming another study [6]) to ~60% (brain) and 40–50% (muscle, liver, kidney). Recently, Pike et al. (7), using aqueous shift reagents to distinguish intra- and extracellular K^+ in perfused heart, calculated that <20% of intracellular K was NMR visible, a figure even lower than that reported for the tissues studied here.

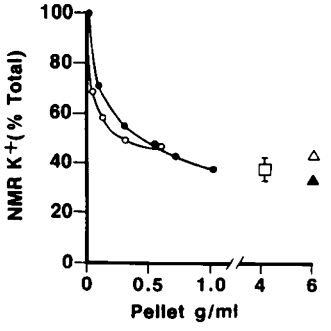

FIGURE 6 The effect of liver homogenate on NMR potassium visibility. *Open circles* and *closed circles*, dilutions of homogenate in 150 mM KCl solution. *Open square*, the mean of six undiluted homogenate; *open triangles* and *closed triangles*, concentrated liver homogenate pellets, prepared by centrifugation at 50,000 *g* for 2 h and removal of the supernatant.

The ^{39}K nucleus has a spin 3/2 and thus possesses a quadrupole moment. ^{23}Na also has a quadrupole moment and has been extensively studied by NMR in intact tissue, providing insight into the mechanism of the varying NMR visibility of ^{39}K. Original studies comparing Na^+ detected by NMR and flame photometry found that ~40% of the Na^+ was detectable (11), this being interpreted to indicate that 60% of the Na^+ was "bound," broadening the NMR signal beyond detection (13). However, Berendsen and Edzes (14) and Civan and Shporer (15, see also reference 8) pointed out that transient immobilization of a small amount of cell Na^+ or diffusion of Na^+ through a highly anisotropic environment could lead to a broadening of the $\pm 3/2$ to $\pm 1/2$ transition, making only the $+1/2$ to $-1/2$ transition detectable. Since the $+1/2$ to $-1/2$ transition represents 40% of the total intensity one would expect to see only 40% of the total Na^+ even though an insignificant fraction of the Na^+ was "bound." This hypothesis was supported by measurements of nuclear relaxation times (14, 15). Recently studies utilizing aqueous shift reagents to separate intra- and extracellular Na^+ have produced conflicting results. In red blood cells (16) and isolated kidney tubules (17) 100% of the intracellular sodium was NMR detectable. By contrast <20% of the intracellular sodium of perfused hearts is detectable (7), suggesting that Na^+ visibility may not be simply explained by quadrupolar interactions (14, 15).

In the case of K^+, >98% of total tissue K is intracellular so that contributions from extracellular K are negligible. As the amount of detectable K in liver (intact and homogenate), muscle, and kidney is around the 40% level, the signal may well be influenced by transient interactions of the quadrupolar nucleus with its environment.

However, a number of the observations presented here and elsewhere (7) suggest that factors other than transient effects of quadrupolar relaxation are important in NMR measurements of tissue K^+. First, the ^{39}K NMR signal visibility can be as low as 20% in heart (7) and K-depleted muscle, significantly less than the 40% visibility expected from loss of signal due to quadrupolar interactions (14, 15). These results suggest there may be an intracellular compartment in which K^+ is <40% visible by NMR. Second, induced changes in K^+ determined by NMR agree with changes in tissue K^+ content determined by flame photometry (Table II). That is, NMR measures 100% of the potassium added to the cell with acute potassium loading and 100% of the potassium lost from the cell with potassium depletion. This suggests that there is an intracellular compartment in which K^+ is 100% visible by NMR. Finally, addition of liver homogenate to a solution of KCl reduces the NMR signal to 40% of control, indicating that

a large portion of cell K^+ may be 40% visible due to transient effects.

Less convincing, but additional, evidence for explanations other than quadrupolar interactions influencing the K^+ signal are:(*a*) the increased visibility of K^+ in liver homogenates with addition of RbCl is consistent with shift of potassium from a less visible to a more visible site, (*b*) the loss of 60% of the K^+ signal with only a 1:3 dilution of liver homogenate suggests that variations in cell water content of up to 30% could not explain variations in visibility, and (*c*) the wide variety of K^+ visibility in different tissues.

Based on these considerations, a working model for the distribution of K^+ in the cell would consist of three compartments of varying K^+ visibility: first, a pool of 100% NMR visibility to explain quantitative agreement between changes in NMR signal and measured tissue K^+ with K^+ loading or depletion. Such a compartment might be equivalent to the intracellular state of red blood cells. This pool may vary in size between 10 and 20% of total K to explain the variations in visibility observed. Second, a compartment of low visibility (0–40%), to explain visibility <40% in K^+ deplete muscle and control heart (7) and the effect of rubidium on K^+ visibility in liver homogenate. This compartment may be ~10–20% of total K^+ content in muscle and liver. Thirdly, a compartment, ~70–80% of total, in which the potassium NMR signal is reduced to 40% of total due to the effects of transient binding (15) or diffusion through an anisotropic environment (14). The combination of these three compartments would explain the observed total visibility of 40–50% in muscle.

Intracellular compartmentation of K^+ is also consistent with microelectrode studies of intracellular K^+, where some 10–20% less than total K^+ is observed (4). One possible site for a compartment of K^+ where NMR visibility may be altered is within mitochondria; NMR visibility of phosphate in mitochondria has been reported to be <100% (18). If mitochondrial K^+ were an important consideration in NMR K^+ visibility, this would help explain the high visibility of potassium by NMR in mitochondria-free red blood cells.

In summary, these studies indicate a potential for ^{39}K NMR to measure changes in intracellular potassium status, either for physiological or clinical studies. In particular, the ability to quantitate bodily potassium depletion by relating the potassium NMR signal to the proton signal may provide a clinically useful, non-invasive technique for assessing potassium status. Finally, the current results suggest that K^+ may reside within three intracellular compartments. ^{39}K NMR is a unique non-invasive tool to investigate the factors that regulate intracellular K^+ distribution.

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REFERENCES

1. Gabow, P., and I. N. Peterson. 1980. Disorders of potassium metabolism. *In* Renal and Electrolyte Disorders. R. W. Schrier, editor. Little, Brown & Co. Inc., Boston. 183–221.
2. Kernan, R. P. 1980. Cell Potassium. J. Wiley & Sons, Inc., New York. 20–30.
3. MacKnight, A. D. C. 1980. Comparison of analytical techniques: chemical, isotopic and microprobe analysis. *Fed. Proc.* 39:2881–2887.
4. Civan, M. M. 1978. Intracellular activities of sodium and potassium. *Am. J. Physiol.* 234:F261–F269.
5. Ogino, T., J. A. den Hollander, and R. G. Shulman. 1983. ^{39}K, ^{23}Na, and ^{31}P NMR studies of ion transport in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 80:5185–5189.
6. Brophy, P. J., M. K. Hayer, and F. G. Riddell. 1983. Measurement of intracellular potassium ion concentrations by NMR. *Biochem. J.* 210:961–963.
7. Pike, M. M., J. C. Frazer, D. F. Dedrick, J. S. Ingwall, P. D. Allen, C. S. Springer, and T. W. Smith. 1985. ^{23}Na and ^{39}K nuclear magnetic resonance studies of perfused rat hearts. Discrimination of intra- and extracellular ions using a shift reagent. *Biophys. J.* 48:159–173.
8. Civan, M. M. 1983. Applications of NMR spectroscopy to intracellular immobilization and bioenergetics. *In* Epithelial Ions and Transport. Application of Biophysical Techniques. John Wiley & Sons, Inc., New York. 64–92.
9. Koretsky, A. P., S. Wang, J. Murphy-Boesch, M. P. Klein, T. L. James, and M. W. Weiner. 1983. ^{31}P NMR spectroscopy of rat organs, in situ using chronically implanted radiofrequency coils. *Proc. Natl. Acad. Sci. USA.* 80:7491–7495.
10. Thulborn, K. R., and J. J. Ackerman. 1983. Absolute molar concentration by NMR in inhomogeneous B_1. A scheme for analysis of in vivo metabolites. *J. Mag. Reson.* 55:357–371.
11. Kunin, A. R., E. H. Dearborn, and A. S. Relman. 1959. Effect of infusion of rubidium chloride on plasma electrolytes and the electrocardiogram of the dog. *Am. J. Physiol.* 197:231–235.
12. Monoi, H. 1974. Nuclear magnetic resonance of tissue ^{23}Na. I. ^{23}Na signal and Na^+ activity in homogenate. *Biophys. J.* 14:645–651.
13. Cope, F. W. 1965. Nuclear magnetic resonance evidence for complexing of sodium ions in muscle. *Proc. Natl. Acad. Sci. USA.* 54:225–227.
14. Berendsen, H. J. L., and H. T. Edzes. 1973. The observation and general interpretation of sodium magnetic resonance in biological material. *Ann. NY Acad. Sci.* 204:459–485.
15. Civan, M. M., and M. Shporer. 1978. NMR of sodium-23 and potassium-39 in biological systems. *In* Biological Magnetic Reso-

nance. Vol. I. L. J. Berliner and J. Reuben, editors. Plenum Publishing Corp., New York. 1–32.
16. Pike, M. M., E. T. Fossel, T. W. Smith, and C. S. Springer. 1984. High-resolution ^{23}Na-NMR studies of human erythrocytes: use of aqueous shift reagents. *Am. J. Physiol.* 246:C528–C536.
17. Gullans, S. R., M. J. Avison, T. Ogino, G. Giebisch, and R. G. Shulman. 1985. NMR measurements of intracellular sodium in the rabbit proximal tubule. *Am. J. Physiol.* 249:F160–F168.
18. Wang, G. G. 1981. Nuclear magnetic resonance of intact tissues. Ph.D. thesis. Oxford University, Cambridge. 93–117.