^{23}Na AND ^{39}K NUCLEAR MAGNETIC RESONANCE STUDIES OF PERFUSED RAT HEARTS

Discrimination of Intra- and Extracellular Ions Using a Shift Reagent

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ABSTRACT High-resolution ^{23}Na and ^{39}K nuclear magnetic resonance (NMR) spectra of perfused, beating rat hearts have been obtained in the absence and presence of the downfield shift reagent Dy(TTHA)$^{3-}$ in the perfusing medium. Evidence indicates that Dy(TTHA)$^{3-}$ enters essentially all extracellular spaces but does not enter intracellular spaces. It can thus be used to discriminate the resonances of the ions in these spaces. Experiments supporting this conclusion include interventions that inhibit the Na^+/K^+ pump such as the inclusion of ouabain in and the exclusion of K^+ from the perfusing medium. In each of these experiments, a peak corresponding to intracellular sodium increased in intensity. In the latter experiment, the increase was reversed when the concentration of K^+ in the perfusing medium was returned to normal. When the concentration of Ca^{2+} in the perfusing medium was also returned to normal, the previously quiescent heart resumed beating. In the beating heart where the Na^+/K^+ pump was not inhibited, the intensity of the intracellular Na^+ resonance was <20% of that expected. Although the data are more sparse, the NMR visibility of the intracellular K^+ signal appears to be no more than 20%.

INTRODUCTION

The highly diagnostic and nondestructive nature of nuclear magnetic resonance (NMR) spectroscopy provides potentially useful tools to study the involvement of metal cations in cardiac physiology and pathophysiology. Intracellular calcium (Ca_i) is, in many respects, the most crucial cation in this regard (Chapman, 1983). However, Ca NMR, in a system as complex as the intact heart, lacks current practicability for three major reasons. The magnetic isotope, ^{43}Ca, is very rare (and thus expensive); it has a weak receptivity; and since a large fraction of Ca_i is thought to be bound to macromolecular sites, there is a real possibility of significant NMR invisibility of this quadrupolar nucleus (Forsén, 1978). The ^{23}Na nucleus, on the other hand, is 100% abundant, has a reasonably strong receptivity, and though ^{23}Na is also quadrupolar with an even larger linewidth factor the Na^+ ion is expected to experience less binding than Ca^{2+}. Thus, the ^{23}Na NMR study of biological tissue has much precedent (Forsén, 1978; Civan and Shporer, 1978).[1]

Although most Na^+ in tissue is extracellular, the transsarcolemmal Na^+ gradient is an important component supporting the state of excitability of the heart cell (Chapman, 1983). Also in heart, Ca flux and cellular content is intimately coupled to the sodium gradient by a Na^+/Ca^{2+} exchange mechanism across the sarcolemmal membrane (Chapman, 1983). In addition, there is an important Na_i-dependent Ca^{2+} efflux from myocyte mitochondria (Chapman, 1983). Thus the Na^+ gradient is crucial to cardiac homeostasis and performance, and its quantitation is of considerable interest. Recent progress has been made in model systems by the use of ion-selective microelectrodes (Chapman, 1983), but sampling problems limit the effectiveness of this approach in estimating Na^+ activity gradients across the sarcolemmal membrane of the intact functioning heart.

Until recently, ^{23}Na NMR studies of tissue suffered from the inability to distinguish between intra- and extracellular Na^+. However, the introduction of aqueous hyperfine shift reagents for cationic NMR (Pike and Springer, 1982; Gupta and Gupta, 1982) offered a potential solution to this problem. These shift reagents, anionic chelates of

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[1] *Abbreviations used in this paper:* PPP^{5-} is tripolyphosphate, $P_3O_{10}^{5-}$; $TTHA^{6-}$ is triethylenetetraminehexaacetate; $DTPA^{5-}$ is diethylenetriaminepentaacetate; $EDTA^{4-}$ is ethylenediaminetetraacetate; Tris is $H_2NC(CH_2OH)_3$.

paramagnetic lanthanide ions, are not expected to cross normal cell membranes and have been used to discriminate intra- and extracellular Na^+, K^+, and/or Li^+ in suspensions of human erythrocytes (Gupta and Gupta, 1982; Gupta et al., 1982; Brophy et al., 1983; Pettegrew et al., 1984; and Pike et al., 1984), yeast cells (Balschi et al., 1982; Ogino et al., 1983) and in preparations of frog muscle (Gupta and Gupta, 1982) and frog skin (Civan et al., 1983; Balaban, 1983). The shift reagent $Dy(PPP)_2^{7-}$ introduced by the Guptas (Gupta and Gupta, 1982) produces the largest shifts of any known shift reagents of this type (Chu et al., 1984). However, it is quite sensitive to the presence of Ca^{2+} or Mg^{2+} and the shifts it produces are decreased considerably by these ions. This may be due to simple competition for the monovalent ion binding site on the shift reagent or the stripping of the PPP^{5-} ligand from Dy^{3+} by the divalent ion (Chu et al., 1984). One can protect the shift reagent by the addition of excess PPP^{5-}, which chelates Ca^{2+} and Mg^{2+} (Civan et al., 1983). However, in a tissue as acutely sensitive to Ca_o as the heart (Chapman, 1983), one could only protect the shift reagent at the expense of the physiologic function of the tissue. Excess Ca^{2+} would protect the heart at the expense of the effectiveness of the shift reagent. Although it produces smaller shifts, the $Dy(TTHA)^{3-}$ ion is more Ca^{2+} and Mg^{2+} tolerant (Chu et al., 1984). It has a large stability constant ($\sim 10^{20}$ [Chu, et al., 1984]) and is likely to be less toxic than $Gd(DTPA)^{2-}$, which has been used as a contrast reagent for the NMR imaging of living animals with few, if any, harmful effects (Weinmann et al., 1984; Brasch et al., 1984; Runge et al., 1983). Also, $Dy(DTPA)^{2-}$ has been used as a susceptibility relaxation reagent in NMR studies of human erythrocytes (Brindle et al., 1979). For these reasons, we have perfused intact, beating rat hearts with buffered media containing $Dy(TTHA)^{3-}$ in a series of experiments designed to test the hypothesis that this shift reagent can be used to discriminate between intra- and extracellular monovalent cations in the intact, functioning heart using ^{23}Na and ^{39}K NMR.

EXPERIMENTAL

Shift Reagent

Stock solutions of $Dy(TTHA)^{3-}$ were prepared from $DyCl_3 \cdot 6H_2O$ (Alpha Products, Danvers, MA) and H_6TTHA (Sigma Chemical Corp., St. Louis, MO) according to published procedures (Pike and Springer, 1982; Chu et al., 1984; Pike et al., 1983) in two forms: the sodium salt, $Na_3Dy(TTHA) \cdot 3NaCl$, and the sodium-free salt, $(TrisH)_3Dy(TTHA) \cdot 3TrisHCl$. A slight excess of $TTHA^{6-}$ ($TTHA^{6-}/Dy^{3+}$, mole ratio = 1.01) was used to preclude inadvertent excess Dy^{3+}. Other chemicals and drugs were used as obtained: H_2EDTA^{2-}, Tris, and ouabain (Sigma Chemical Corp.), and verapamil (Calan, G. D. Searle, Skokie, IL). Glass-distilled (and deionized) water was used throughout.

Bathing Solutions

Two types of solutions were used to bathe the heart preparations. One contained 310 mM mannitol (Sigma Chemical Corp.). The other contained ~290 mM mannitol and either 10 or 15 mM $(TrisH)_3Dy(TTHA) \cdot 3TrisHCl$.

Perfusing Solutions

Two types of perfusing solutions were used. One was a standard Krebs-Henseleit (K-H) buffer solution containing 121 mM NaCl, 23 mM $NaHCO_3$, 5.9 mM KCl, 1.75 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.5 mM Na_2H_2EDTA, and 11.0 mM glucose at pH = 7.4. The other contained $Na_3Dy(TTHA) \cdot 3NaCl$ in addition to the other constituents. The NaCl concentration was adjusted so that the total Na^+ concentration remained 145 mM. If the shift reagent was 10 mM, the $CaCl_2$ concentration was raised to 3.0 mM (2.6 mM, if the H_2EDTA^{2-} was not present) in order to maintain normal cardiac performance (see Results). If the shift reagent was 15 mM, the $CaCl_2$ concentration was raised to 4.2 mM. For some experiments, the KCl and/or $CaCl_2$ concentrations were lowered as described in the Results section.

Heart Preparation and Perfusion

Perfused hearts were prepared essentially as described by Ingwall (Ingwall, 1982). Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 350–500 g were anesthetized with intraperitoneal pentobarbital (Abbott Laboratories, Diagnostic Div., Irving, TX). The hearts, weighing 1.6–2.1 g, were quickly removed and placed in physiologic medium (K-H buffer) at 4°C to arrest the heart. Excess tissue (lung, bronchus, esophagus, thymus gland, and pericardium) was removed. Within 1 min, the aortic root was secured on a cannula attached to the perfusion apparatus (Ingwall, 1982) and perfusion via the coronary arteries was initiated. The apparatus was fitted with two separate chambers and reservoirs so that the perfusing medium could be switched rapidly from one buffered solution to another. Each chamber, reservoir, and attached transfer line was water-jacketed and thermostated at 37°C. The chambers were vigorously bubbled with 95:5 O_2/CO_2 gas. A final water-jacketed umbilical led the various tubes to the perfusion head that contained the windkessel and that fit snugly into a 20-mm NMR tube. The entire perfusion apparatus was mounted on a movable cart and the perfusion chambers were placed at the appropriate height to provide 100 mmHg hydrostatic pressure at the aorta when the NMR tube was at its proper depth in the top-loading superconducting magnet. Buffer solution flowing down the aortic line passed through the windkessel, placed just above the heart, which served as both a bubble trap and a compliance chamber.

Three types of rat heart preparations were used. In the working heart preparation ($N = 4$), the heart was cannulated at the pulmonary vein (left atrium) (10–15 mmHg perfusion pressure) in addition to the aorta, so that the left heart functioned as it does in vivo. The pressure changes for the working heart were detected at the lower end of the windkessel, where a direct line led to a pressure transducer. The pressure tracing was recorded with a Hewlett Packard 7754B recording system (mean systolic/diastolic pressure 160/75 mmHg [$N = 2$]; heart rate in one heart 280 beats min^{-1}) (Hewlett-Packard Co., Palo Alto, CA). In the isovolumic Langendorff preparation ($N = 12$) the heart was cannulated at the aorta only. A latex balloon on the tip of a small polyethylene tube (Intramedic, Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, NJ) was inserted into the left ventricle through the left atrium. The balloon was filled with ~0.2 ml of aqueous medium, usually containing sodium-free shift reagent, and was in direct fluid contact with the pressure transducer (mean systolic/diastolic pressure 147[±21]/13[±8]; quantities given in square brackets are standard deviations [$N = 11$]; mean heart rate = 260[±25] beats min^{-1} [$N = 11$]). The recirculating (~1.5 l) preparation used for one heart was identical to the isovolumic Langendorff preparation, except that the pulmonary artery (right ventricle) was also cannulated by a flexible aspiration line, with a tiny air vent just above the heart, to lead the effluent back to the lower reservoir. Suction was applied to the aspiration line at a flow rate much faster than the coronary flow; the air vent prevented a negative pressure from developing in the heart but still

allowed adequate aspiration (systolic/diastolic pressures 135/45; heart rate, 280 beats min^{-1}).

In all the heart preparations, the heart was bathed with aqueous medium for better magnetic susceptibility matching of the heart and the surrounding space. In most cases, a sodium-free aqueous isotonic mannitol bathing solution was piped via thin polyethylene tubing (Intramedic, Clay Adams, Div. of Becton, Dickinson & Co.) to the bottom of the NMR sample tube at a flow rate comparable to the coronary flow. Both the mannitol bathing solution and the perfusate were aspirated out of the tube above the heart. In the recirculating preparation, the perfusate was aspirated directly from the pulmonary artery and the mannitol bath was aspirated at a point above the heart. The coronary flow, the flow of the bathing medium, or their sum was measured periodically by timing the accumulation of a volume of effluent in a graduated cylinder. The average coronary flow in eight isovolumic Langendorff hearts was 20(±4) ml min^{-1}; in two working hearts, it was 17 ml min^{-1}; and in the recirculating heart preparation it was 22 ml min^{-1}.

NMR Spectroscopy

The instrument used was a Nicolet wide-bore 360 (^{1}H)MHz NMR spectrometer (8.46 T) (Nicolet Magnetics Corp., Fremont, CA) equipped with sodium (^{23}Na, 95.245 MHz) and broad-band potassium (^{39}K, 16.8 MHz) probes accepting 20-mm sample tubes. A dual probe containing two transmitter/receiver Helmholtz radio frequency coils was also used. One coil was double tuned to the ^{23}Na and ^{2}H (55.3 MHz) resonances; the other was tuned to phosphorus (^{31}P, 145.8 MHz) (Höfeler et al., 1984; Höfeler, H., J. A. Balchi, D. Jensen, M. Pike, V. P. Cirillo, J. Delayre, C. S. Springer, and E. T. Fossel, manuscript in preparation). The probes were fine-tuned to the desired resonance with the hearts in place. Most ^{23}Na signals were acquired with a single 90° pulse (~40 μs) followed by an acquisition tme of 0.256 s. This was longer than five times the longest longitudinal relaxation time (T_1) value in the spectrum (see below). Sodium spectra with negligible noise could be taken in 12.3 s (48 transients), thus defining the time resolution of the experiments. The kinetic mode (Nicolet Magnetics Corp.) of data collection was often used, in which free-induction decays (FID) was produced, accumulated, and stored at preprogrammed intervals automatically. In most cases, the ^{23}Na FID was multiplied by a factor with an exponential time constant appropriate for sensitivity enhancement (line broadening [LB]; typically, LB = 3 Hz) before Fourier transformation. The inversion recovery technique was used to measure the values of T_1 for the extracellular Na^+ resonance (35 ms) and the intracellular Na^+ resonance (25 ms) observed after the steady state effects of 10-mM shift reagent and ouabain were attained.

Differences between transformed spectra were obtained using the Nicolet AS spectral subtraction subroutine (Nicolet Magnetics Corp.). Portions of difference spectra were integrated using the standard Nicolet ID integration subroutine (Nicolet Magnetics Corp.). Some FID were multiplied by a function appropriate for resolution enhancement (double exponential multiplication [DM]) before transformation. However, spectra obtained after this treatment were not used for integration. In some experiments, a pulse sequence ($90°_y, \tau, 90°_{-y}$) (Plateau and Guéron, 1982; Plateau et al., 1983; and Hore, 1983) was used to suppress most of the intensity of the large extracellular resonance so that the small intracellular resonance could be more readily quantitated.

RESULTS

Fig. 1 shows a stacked plot of ^{23}Na NMR spectra of an isovolumic Langendorff-perfused rat heart and a portion of the simultaneous P vs. t record. The first spectrum is that of the heart perfused with the standard K-H solution. The heart was continuously bathed with sodium-free isotonic mannitol solution 10 mM in the downfield hyperfine shift reagent $Dy(TTHA)^{3-}$. The small peak 215 Hz (2.26 ppm) downfield represents a small amount of Na^+ in the portion of the bath inside the receiver coil. This signal shifts back into the main peak if the shift reagent is removed from the bathing solution (not shown). This Na^+ must derive from the small portion of the coronary effluent that diffuses into the lower regions of the bath or ions that are leached from the heart by the flowing bath. Experiments with the dye methylene blue in the perfusing medium indicated that most of the coronary effluent was quickly aspirated from the top of the bath with the mannitol solution flowing. If the flow of a shift reagent-free mannitol bathing solution is shut off and the heart is bathed in its own perfusate, the intensity of the single sharp signal increases by as much as 150%.

The major sharp resonance in the first spectrum (linewidth 45 Hz without resolution enhancement) thus represents Na^+ inside the heart. The shoulder on the downfield side of the resonance is caused by the presence of Na^+-free shift reagent in the intraventricular balloon, probably due to a bulk magnetic susceptibility shift of some heart Na^+ signals. With no shift reagent in the balloon, a peak with a linewidth of 30 Hz could often be obtained. The first spectrum in Fig. 1 is representative of those obtained during the prior 17-min period that the heart was perfused with the shift reagent-free buffer solution. After that, the spectra are marked with the number of minutes that have elapsed (at the midpoint of the acquisition period) since 30 s after the perfusing medium was switched to that containing 10 mM $Dy(TTHA)^{3-}$. (It was determined from the P vs. t record that ~30 s was required for a new medium to reach the heart from the switching valve.)

The effect on the spectrum of perfusing the heart with medium containing the shift reagent is both dramatic and swift. Within a few seconds, major portions of the Na^+ signal are shifted downfield, ultimately by more than 3 ppm. By the time 2 min have elapsed, most of the change has been completed. This can be seen more clearly in Fig. 2, where some of the spectra from Fig. 1 are shown in both sensitivity-enhanced and resolution-enhanced forms. A plot of the peak height at the unshifted position as a function of time after shift reagent entry is shown in Fig. 3. If the shift reagent was washed out of the heart by returning to standard perfusing medium (not shown), the spectrum recovered its initial appearance, with a single sharp resonance at the unshifted position.

Apart from minor, transient changes in the P vs. t record (of the sort shown in Fig. 1), the hearts tolerated the introduction of $Dy(TTHA)^{3-}$ well. Typically, when isovolumic Langendorff-perfused rat hearts were exposed to 10 mM $Dy(TTHA)^{3-}$, the heart rate decreased from 260 beats min^{-1} (see Experimental) to 220(±23) beats min^{-1} (N = 6), the ventricular pressures remained stable (147/13 mmHg [see Experimental] to 151[±27]/14[±11] mmHg [N = 10]), and the coronary flow changed insignificantly (20[±4] ml min^{-1} [see Experimental] to 17[±4] ml min^{-1} [N = 3]). As explained in the Experimental section, the

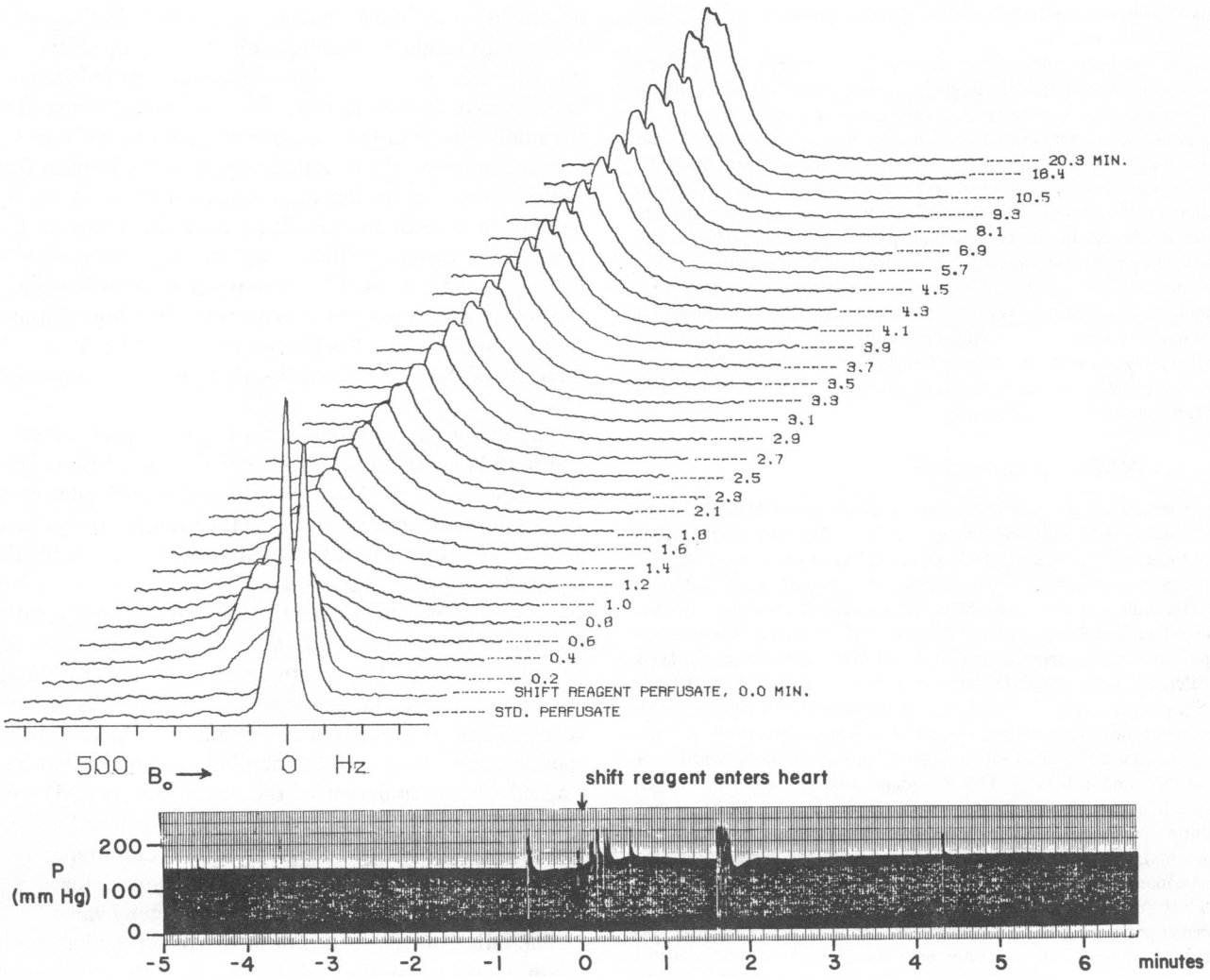

FIGURE 1 ^{23}Na NMR spectra (95.2 MHz, 8.5 T) of an isovolumic Langendorff-perfused beating rat heart. The first spectrum was obtained near the end of a 17-min period during which the heart was perfused with the standard medium and the physiologic parameters were: heart rate, 230 beats min^{-1}; systolic/diastolic pressures 145/15; coronary flow, 12 ml min^{-1}. Spectral acquisition parameters are given in the text. Subsequent spectra are labeled with the times (midpoints of acquisitions) elapsed since a perfusion medium containing 10 mM $Dy(TTHA)^{3-}$ entered the heart. After 26 min of this perfusion the pressures were 170/20. The spectra displayed have been resolution enhanced with a DM value of 10. The portion of the P vs. t trace recorded during shift reagent wash-in is displayed below the spectra.

concentration of Ca^{2+} was raised from 1.75 to 3.0 mM when $Dy(TTHA)^{3-}$ was introduced into the perfusing medium. This was empirically determined (from the P vs. t trace) to compensate for the Ca^{2+} bound by $Dy(TTHA)^{3-}$ (Chu et al., 1984).

A downfield shift of the heart Na^+ NMR signal is expected as $Dy(TTHA)^{3-}$ enters various Na^+-containing compartments in the heart. Perhaps surprisingly, after the heart achieved its steady-state level of shift reagent, there was almost no observable signal remaining at the unshifted position (Fig. 3). The simplest explanation for this observation is that $Dy(TTHA)^{3-}$ enters all or almost all Na^+-containing compartments in the heart. However, we had no evidence from prior studies with human erythrocytes (Pike et al., 1984) or yeasts (Höfeler et al. 1984; Höfeler, H., J. A. Balschi, D. Jensen, M. Pike, V. P. Cirillo, J. Delayre, C. S. Springer, and E. T. Fossel, manuscript in preparation) that $Dy(TTHA)^{3-}$ could cross normal cell membranes and enter the intracellular milieu. It is of interest that the time course seen in Fig. 3 is very similar to that observed for the uptake of $^{58}Co(EDTA)^-$ by perfused rabbit hearts by Bassingthwaighte and co-workers (Bridge et al., 1982). These investigators showed that although $Co(EDTA)^-$ leaves the vasculature and enters the interstitial space, it remains extracellular. Also, Weinmann and co-workers concluded that $Gd(DTPA)^{2-}$ is "distributed exclusively extracellularly" when delivered intravenously to live rats (Weinmann et al., 1984). The remaining experiments of this study were designed to demonstrate that this was also true for $Dy(TTHA)^{3-}$ in the intact perfused rat heart.

Since K^+, in contrast to Na^+, is concentrated inside

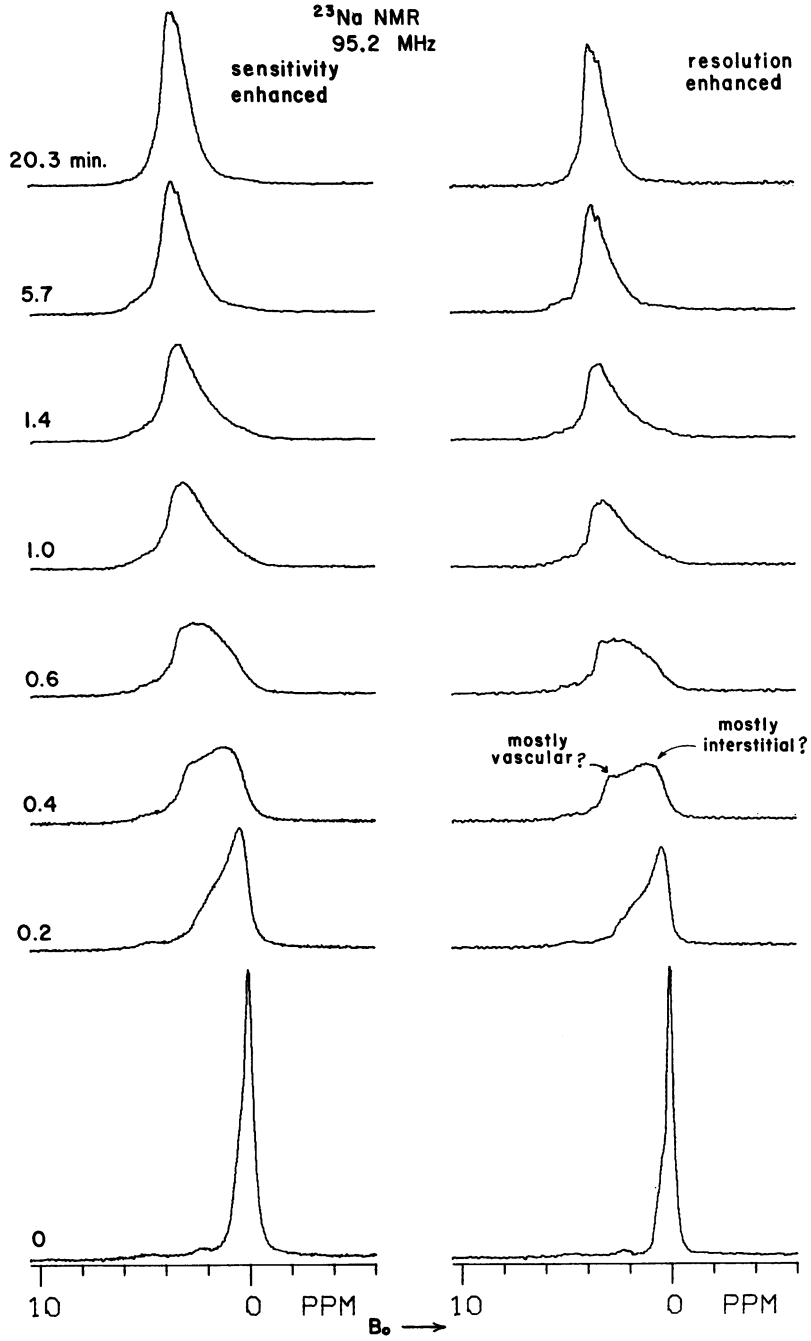

FIGURE 2 Selected spectra from those of Fig. 1. The spectra on the left have been sensitivity enhanced with a LB of 3 Hz. Those on the right are resolution enhanced with a DM value of 10. The labels correspond to those of Fig. 1.

myocardial cells, a ^{39}K NMR spectrum in the presence of shift reagent is more likely to exhibit an intracellular resonance. Fig. 4 *A* shows a ^{39}K NMR spectrum of a working rat heart. The heart was first perfused with standard medium and without the mannitol bathing solution flowing. In the spectrum in Fig. 4 *B*, the midpoint of the accumulation occurred 7 min after the perfusing medium was switched to one containing 8.5 mM $Dy(TTHA)^{3-}$ (and sufficient extra Ca^{2+} [~1.5 mM] so that free Ca^{2+} was 1.75 mM by ion-specific electrode). The large peak near the unshifted position is reasonably assigned to intracellular K^+ (K_i). A downfield bulk susceptibility shift of <0.8 ppm (analogous to that reported below for Na_i) could not be easily detected on the spectral scale of Fig. 4. The position and intensity of this peak strongly indicate that $Dy(TTHA)^{3-}$ has, at least, not entered a major portion of the intracellular compartments. Although a considerable amount of spectral intensity has shifted in this experiment, this is expected because the heart is bathed in its own effluent perfusate without a separately

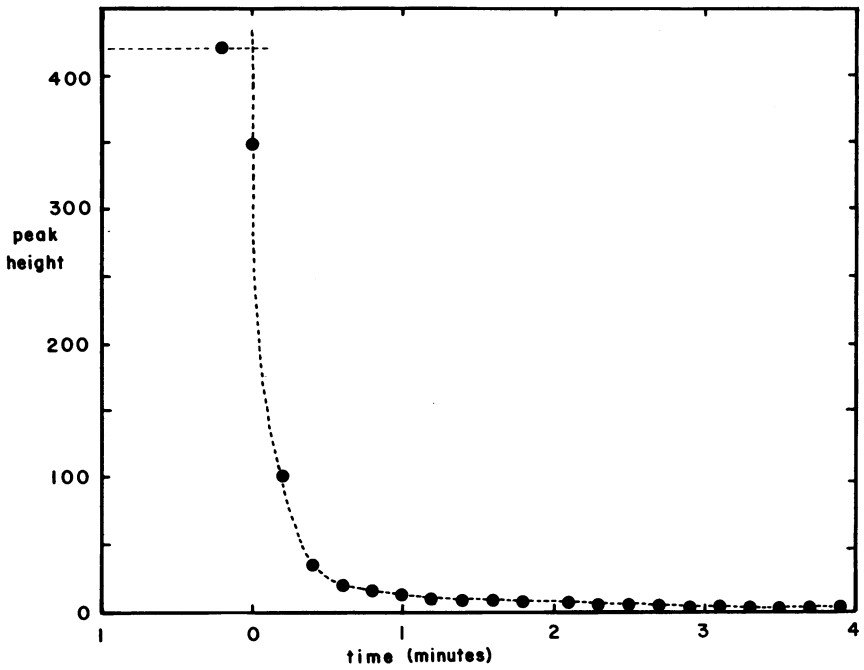

FIGURE 3 The spectral intensity (peak height, in arbitrary units) at the zero hertz (parts per million) position as a function of time after shift reagent enters the heart of Figs. 1 and 2. The zero position is that of the peak obtained in the perfusion with standard medium. The dashed lines are intended only to guide the eye.

flowing bath. When the mannitol flow was turned on, the intensity of the downfield peak was reduced (Fig. 4 *C*). Curve resolution and integration of the resulting peaks revealed the relative areas of the shifted and unshifted signals to be 36 and 64, respectively. Fig. 4 *D* shows the spectrum of the same heart 61 min after ischemia was initiated by simultaneously stopping the perfusing and bathing flows.

Ouabain is a cardiac glycoside that is a specific inhibitor of the sarcolemmal Na^+/K^+ pump and is known to allow intracellular Na^+ to rise as Na^+ efflux is inhibited and influx continues. Fig. 5 depicts a stacked plot of ^{23}Na NMR spectra of an isovolumic Langendorff-perfused heart obtained during 23 min of perfusion with a medium containing 10 mM $Dy(TTHA)^{3-}$ and 1 mM ouabain. Before the introduction of ouabain, the heart had been perfused for 30 min with 10 mM $Dy(TTHA)^{3-}$. Since ouabain is quite toxic at 1 mM, the *P* wave amplitude decreases until the heart stops beating ~5 min after onset of perfusion with the drug. The first spectrum was obtained just before ouabain entered the heart. The subsequent spectra are labeled with the number of minutes elapsed since ouabain entered the heart, as measured at the midpoint of each spectral acquisition. A small peak appears near the unshifted positon and it progressively increases in intensity after the introduction of ouabain. This can be seen even more clearly in Fig. 6, in which some of the spectra from Fig. 5 are shown in both sensitivity-enhanced and resolution-enhanced forms. The small shift of the putative intracellular peak (~0.8 ppm from the unshifted position) is probably due to a bulk magnetic susceptibility effect caused by the presence of shift reagent in other compartments.

Spectral alterations following an intervention that causes shift reagent to enter the cells could also be used to support the contention that it does not do so under normal circumstances. A high concentration of H_2EDTA^{2-} reduces free Ca_o^{2+} to the point that sarcolemmal permeability increases markedly, (Zimmerman and Hülsmann, 1966). Fig. 7 depicts a stacked plot of spectra showing the effects of the addition of 6 mM Na_2H_2EDTA to the ouabain-containing perfusing medium of the heart of Figs. 5 and 6. Thus, after the first 23 min of ouabain (identical to Fig. 5), the intracellular resonance decreases in intensity as the H_2EDTA^{2-}-induced entrance of shift reagent to the cells occurs. Also, the intensity of the shifted peak increases disproportionately, probably because the leaky cells gain even more Na^+.

To quantitate the area of the small intracellular ^{23}Na resonance, we have examined a number of methods. We have resolved the small peak from the large extracellular peak by use of the Nicolet CAP subroutine (Nicolet Magnetics Corp.) and then integrated the resolved components. Alternatively, we have subtracted the steady-state spectrum obtained in the presence of shift reagent only from those obtained during ouabain perfusion. The extracellular peaks largely cancel and the small intracellular resonance becomes more obvious. However, we have found most useful a third approach using a pulse sequence intended to suppress the large extracellular resonance. Useful results were previously obtained with the Plateau and Guéron "jump-return" sequence (90_y-τ-90_{-y}, with the

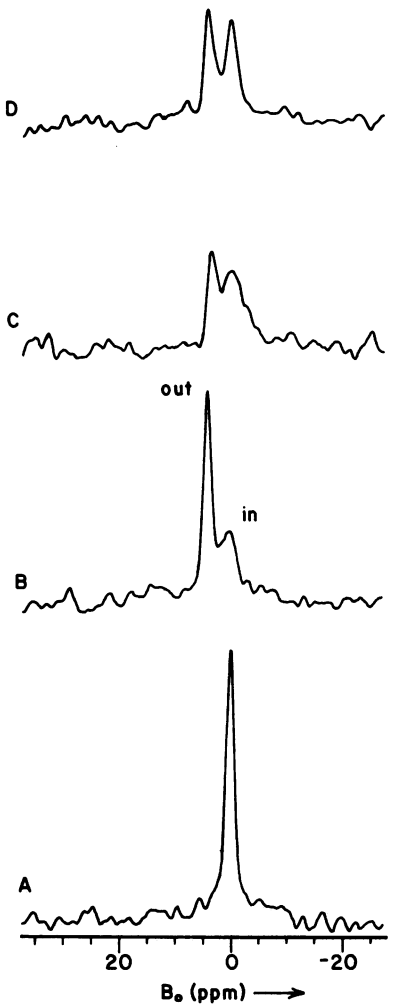

FIGURE 4 ^{39}K NMR spectra (16.8 MHz, 8.5 T) of a perfused working rat heart. Spectrum *A* was obtained during perfusion with the standard medium. The bathing solution was not flowing. The midpoint of the accumulation of spectrum *B* occurred 7 min after the perfusing medium was switched to one containing 8.5 mM $Dy(TTHA)^{3-}$. The bathing solution was not flowing. Spectrum *C* was obtained after the flow of a K^+-free, shift reagent-free bathing solution was started. The midpoint of the accumulation of spectrum *D* occurred 61 min after the perfusing and bathing flows were simultaneously stopped. Each spectrum was the result of 3,000 transients accumulated in 5.13 min. Each accumulated FID was left-shifted, zero-filled, and had undergone a Gaussian multiplication (the GM value was 20) before transformation.

transmitter centered on the peak to be suppressed [Plateau and Guéron, 1982; Plateau et al., 1983; Hore, 1983]) to suppress the large extracellular ^{23}Na resonance in whole blood containing shift reagent in the plasma (Pike, M. M., E. T. Fossel, T. W. Smith, and C. S. Springer, unpublished results). We extended this approach to the isovolumic Langendorff-perfused rat heart as shown in Fig. 8. A jump-return spectrum obtained before the introduction of ouabain is shown in Fig. 8 *A*. The jump-return spectrum in Fig. 8 *B* was obtained after 27 min of perfusion with 1 mM ouabain. In neither case is the large extracellular peak as well suppressed as in the whole blood experiment (Pike, M. M., E. T. Fossel, T. W. Smith, and C. S. Springer, unpublished results). This may be due to its greater width and/or the fact that the transmitter was centered slightly on the upfield side of the downfield peak. When the difference between suppressed spectra in the absence (*A*) and presence (*B*) of ouabain is taken ($C = B - A$), however, the intracellular ^{23}Na resonance stands out quite clearly.

To confirm the assignment of the less shifted resonance to intracellular Na^+, we sought a way to raise the level of Na_i without irreversibly damaging the heart. The sarcolemmal Na^+/K^+ pump can be effectively inhibited by lowering the K_o level (Sperelakis, 1979; Barry et al., 1982). Thus, Fig. 9 shows a stacked plot of jump-return ^{23}Na NMR spectra obtained while an isovolumic Langendorff-perfused heart was perfused with 10 mM $Dy(TTHA)^{3-}$ and zero K_o for 23 min. The level of Ca_o was lowered to 230 μM in order to protect the heart from potential damage due to Ca_i overload (Chapman, 1983). The heart stopped beating. The rise in Na_i can be seen clearly. After 23.5 min, the perfusing medium was returned to normal K_o (5.9 mM) while the Ca_o level was kept at 230 μM for 11 more minutes. The intensity of the less shifted peak recedes as Na_i is pumped out of the intracellular compartments. After restoration of Ca_o to normal (3 mM), the heart resumed beating.

The quantitation of Na_i can be more readily accomplished by taking differences after using the pulse suppression sequence. This is shown in Fig. 10, where various spectra from the experiment of Fig. 9 and their differences are shown. The suppressed spectra in Fig. 10 *A*, *B*, and *C* are those at zero, 23.3 min (Fig. 9), and 41.4 min, respectively. The spectrum in *D* is $C - A$ and that in *E* is $B - A$. The Na_i peak is particularly well seen in *E*.

DISCUSSION

The transsarcolemmal distributions of Na^+ and K^+ are vital to the maintenance and control of cardiac excitability and contractile state (Langer, 1968; Sperelakis, 1979; and Chapman, 1983). Examination of these distributions is a challenging problem, and one that is particularly difficult to address by nondestructive methods. While substantial progress has been made in recent years by the use of ion-sensitive microelectrodes (Thomas, 1978; Fozzard and Shew, 1980; and Dagostino and Lee, 1982), this technique is limited by sampling problems and has not yet been applied to intact, functioning hearts. Therefore, a nondestructive method that could maintain continual surveillance of the transsarcolemmal distribution of Na^+ and K^+ in the intact, beating heart (or other organs) would be of considerable biological interest. We report here the successful application of ^{23}Na and ^{39}K NMR, using a nonpermeant hyperfine shift reagent, to this problem.

The behavior of the *P* vs. *t* trace as $Dy(TTHA)^{3-}$ enters the heart (Fig. 1) serves as good evidence that $Dy(TTHA)^{3-}$ is not particularly toxic to the heart. The ratio of the intensities of the creatine phosphate to inor-

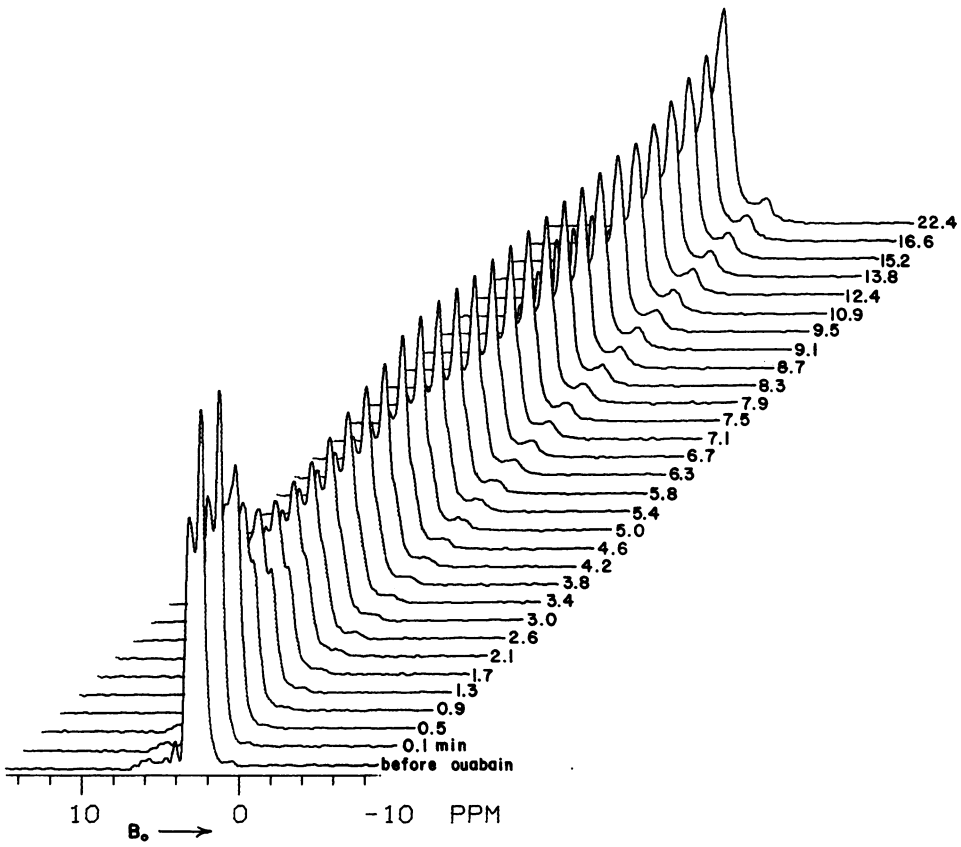

FIGURE 5 ^{23}Na NMR spectra (95.2 MHz, 8.5 T) of an isovolumic Langendorff-perfused beating rat heart. Before the acquisition of these spectra, the heart had been perfused for 29 min with the standard medium. The heart rate was 250 beats min^{-1}, the systolic pressure 175 mmHg, and the coronary flow 24 ml min^{-1}. The first spectrum was obtained at the end of a subsequent 30-min period during which the heart was perfused with a solution containing 10 mM Dy(TTHA)$^{3-}$ and during which the heart rate was 204 beats min^{-1} and the systolic pressure was 175 mmHg. Subsequent spectra are labeled with the times (midpoints of acquisitions) elapsed since a perfusion medium containing 10 mM Dy(TTHA)$^{3-}$ and 1 mM ouabain entered the heart. During the ~23 min of this perfusion, the coronary flow was 20 ml min^{-1}. During all three perfusions, the heart was bathed with a flowing solution containing 10 mM Dy(TTHA)$^{3-}$. Each spectrum was the result of the acquisition of 96 transients except the last (22.4 min), for which only 48 transients were obtained. The vertical scale was adjusted to correct for this. The spectra displayed have been resolution enhanced with a DM value of 20.

ganic phosphate ^{31}P NMR peaks is a sensitive indicator of the vitality of the heart (Ingwall, 1982). We have observed these peaks as the heart is perfused with Dy(TTHA)$^{3-}$. Although the peaks are broadened by magnetic susceptibility effects, the ratio appears to remain the same as in the heart perfused with standard K-H buffer solution. This is confirmed in the normal, sharp ^{31}P NMR spectrum observed during perfusion with medium containing the diamagnetic surrogate Y(TTHA)$^{3-}$. In our hands, the shift reagent Dy(PPP)$_2^{7-}$ (at least with no extra Ca^{2+} added) has proven to be toxic to the perfused, beating rat heart. We have not examined many different Dy(PPP)$_2^{7-}$/Ca^{2+} combinations because of the ubiquity of precipitation in those that we did prepare.

A number of spatial compartments containing Na^+ and K^+ exist in the isolated perfused heart preparation used in these studies. These include the bath, the cardiac chambers (atria and ventricles), the coronary vasculature, the interstitial space, and the intracellular spaces including the subcellular organellar spaces. The small ^{23}Na NMR signal arising from the medium bathing the heart was often shifted downfield farther than those from other compartments containing an equal concentration of shift reagent, when Dy(TTHA)$^{3-}$ was added to the bathing solution (Figs. 1, 2, 5–7). This most likely arises from the lower ionic strength of the bath solution and from its low Na^+ concentration (Pike and Springer, 1982; Chu et al., 1984; Pike et al., 1983). Much of the potential signal from the ventricular chambers was eliminated in the isovolumic Langendorff preparation by the insertion of a balloon containing sodium-free medium in the left ventricle. The remaining ventricular (and atrial) spaces are topologically continuous with the vascular compartment in any case. The presence of 10 mM Dy(TTHA)$^{3-}$-inside the balloon had two effects. The heart ^{23}Na signal shifted slightly downfield (10–20 Hz) with the appearance of a downfield shoulder in the absence of shift reagent elsewhere. This was probably due to a magnetic susceptibility effect. A susceptibility matching effect (Becker, 1980; Brindle et al., 1979; Fabry and San George, 1983) may also occur when

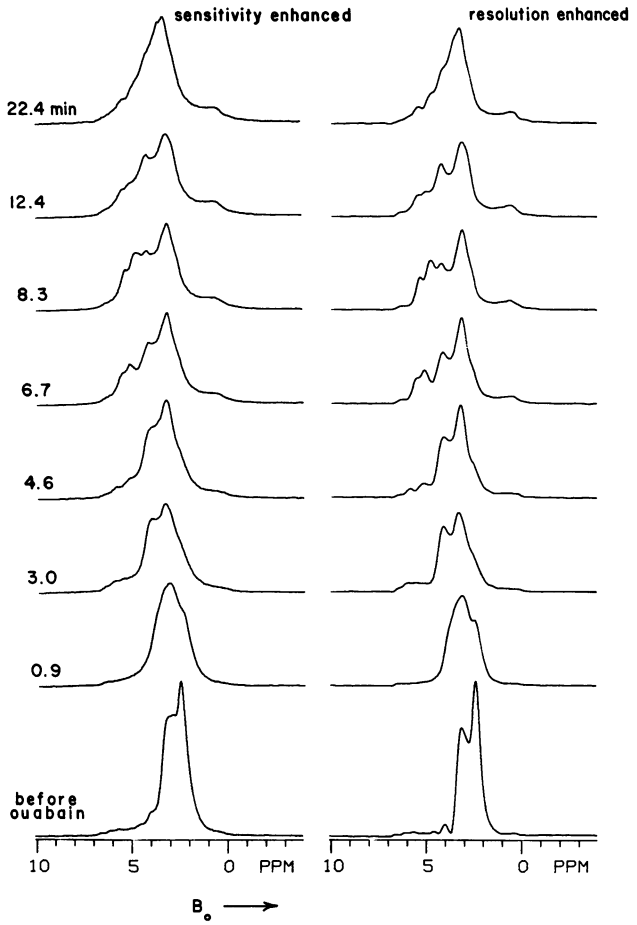

FIGURE 6 Selected spectra from those of Fig. 5. The spectra on the left have been sensitivity enhanced with a LB of 3 Hz. Those on the right have been resolution enhanced with an DM value of 20. The labels correspond to those of Fig. 5.

shift reagent is present in other compartments as well. This should yield sharper resonances. The vascular, interstitial, and intracellular compartments will be discussed below. Since the $Dy(TTHA)^{3-}$ does not enter the myocardial cells, one would not expect to discriminate the sarcoplasmic and organellar populations of Na^+.

One would like to be able to use ^{23}Na or ^{39}K NMR to measure the concentration (c_j) of Na^+ or K^+ in compartment j. However, the area of a ^{23}Na or ^{39}K NMR peak (a_j) representing the compartment is also proportional to a number of other factors (Eq. 1), where V_j is the volume of the compartment

$$a_j \sim c_j \cdot V_j \cdot F_j \cdot v_j. \quad (1)$$

F_j is the relaxation-flow factor for compartment j, and v_j is an NMR-visibility factor for the compartment.

The concentrations of ions in the bath are low when there is irrigant mannitol flow and we have shown that the Na^+ signal is usually shifted sufficiently far away from the unshifted position when $Dy(TTHA)^{3-}$ is present in the bathing medium that this contribution can be ignored or accounted for. The left ventricular cavity is occupied by a balloon filled with a sodium-free solution. We will assume that, at steady state, the solutions in the remaining cardiac chamber spaces, the vasculature, and the interstitial space have the same concentrations of Na^+ and K^+ as the perfusing solution: 145 mM and 5.9 mM, respectively (Tancredi et al., 1975; Bassingthwaighte and Winkle, 1982). The intracellular concentrations of Na^+ and K^+ vary slightly during the cardiac cycle (Mullins, 1981). Even in nonbeating heart tissue, these are difficult to measure accurately (especially Na_i) (Mullins, 1981). Values for Na_i and K_i ranging from 15–33 and 122–160 mM, respectively, have been reported (Sperelakis, 1979). The Na_i values may be high because almost all errors from the measuring procedure would tend to raise this quantity. Mullins uses, as plausible values, 10 mM for Na_i and 150 mM for K_i (Mullins, 1981). These concentrations are based on the total amounts of intracellular Na^+ and K^+.

The sensitive volume monitored by our Helmholtz receiver coil is ~5 ml. The sensitive bath volume is the difference between this and the volume occupied by the heart. The latter is ~2 ml on the average and could be obtained for a given heart from its mass and the specific volume (0.949 ml [gram wet weight]$^{-1}$ for fetal mouse hearts [Ouellette et al., 1983]). The mean values of the volumes of the other compartments in whole rabbit hearts, expressed as fractions of the volume of total water in the heart tissue (0.8 ml g^{-1}), were found to be 0.19, 0.27, and 0.54 for the vascular, interstitial, and intracellular spaces, respectively, by Bassingthwaighte and co-workers (Gonzalez et al., 1980; Gonzalez, F., and J. B. Bassingthwaighte, manuscript in preparation). The vascular volume does not include the chamber blood volumes. For a beating heart, these fluctuate with time. We estimate the mean volume of each ventricular chamber to be 10% of that of the tissue water (the smaller atrial chambers are mostly collapsed at the time of cannulation). Since we have a sodium-free balloon in the left ventricle of the isovolumic Langendorff preparation, we observe no signal from this chamber. Any chamber volume must be added to the vascular volume in our experiment.

In any repetitive pulse NMR experiment, the signal intensity can be affected by the relationship of the pulse recycle time (t_r) and the value of T_1 (Becker et al., 1979). In any pulse flow NMR experiment, the signal intensity can also be affected by the relationships of these parameters to the average (linear) velocity ($\bar{v}$) of the nuclei through the receiver coil. Arnold and Burkhart first combined these considerations in 1965 (Arnold and Burkhart, 1965). Using Bloch's equation, Arnold and Burkhart derived expressions for F (their Eqs. 3, 5, 6, and 7) for 90° flip angles, F_{90}, depending on whether one has slow or fast and plug or laminar flow through a cylindrical cavity inside the receiver coil. The expressions for F_{90} are fairly complicated functions of t_r, T_1, $\bar{v}$, the length (L) of the receiver coil, and the distance (d) the nuclei are allowed to travel in

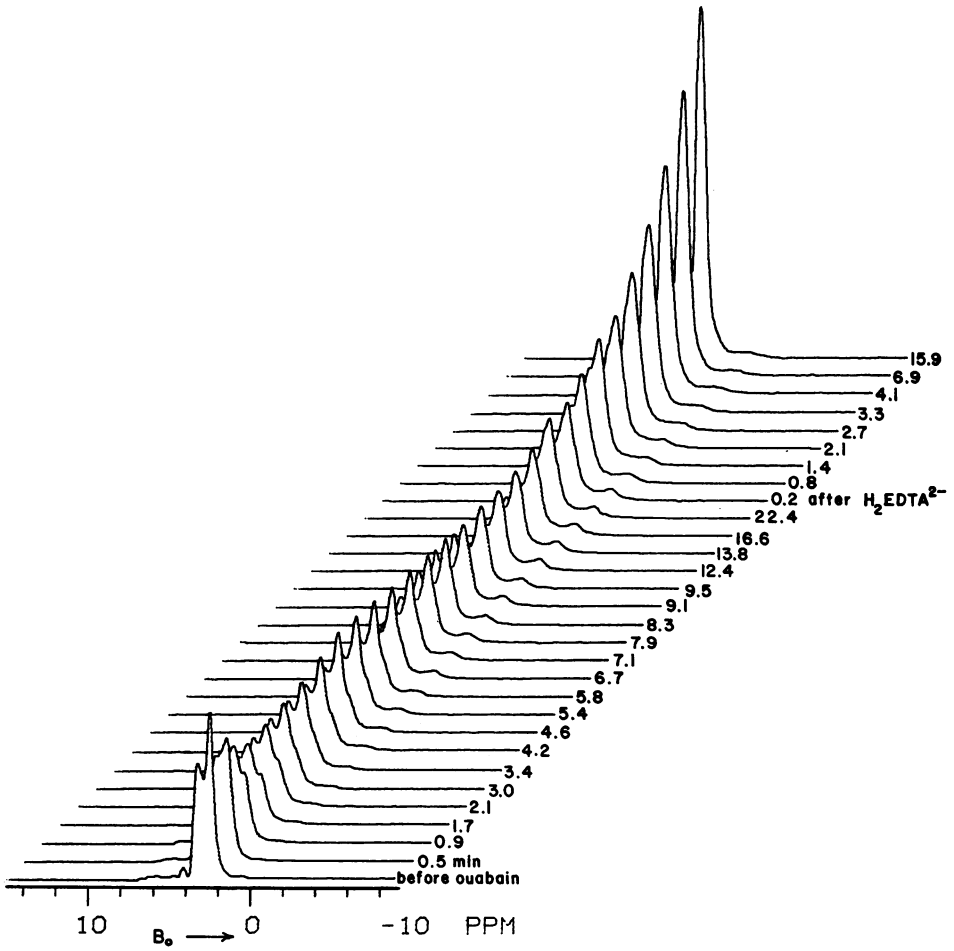

FIGURE 7 ^{23}Na NMR spectra (95.2 MHz, 8.5 T) obtained during a continuation of the experiment of Figs. 5 and 6. The spectra obtained through 22.4 min are the same as those of Fig. 5. Subsequent spectra are labeled with the times (midpoints of acquisitions) elapsed since a perfusion medium containing 10 mM $Dy(TTHA)^{3-}$, 1 mM ouabain, and 6 mM H_2EDTA^- entered the heart. During the ~26 min of this perfusion, the coronary flow was 11 ml min^{-1}. Also, the heart was bathed with a flowing solution containing 10 mM $Dy(TTHA)^{3-}$. Each of these latter spectra was the result of the accumulation of 48 transients and the vertical scales were adjusted to make these comparable to the earlier spectra that resulted from 96 transients. The spectra have been resolution enhanced with a DM value of 20.

the magnetic field before reaching the receiver coil. The result for slow, plug flow is shown in Eq. 2 (where F_{90}, T_1, and $\bar{\nu}$ could be indexed for each compartment). A general result is that

$$F_{90} = [1 - \exp(-t_r/T_1)] \cdot [1 - (\bar{\nu}t_r/L) - (\bar{\nu}T_1/L)\exp(-d/(\bar{\nu}T_1))] + (\bar{\nu}t_r/L). \quad (2)$$

F_{90} can never be greater than one. The increase of F by $\bar{\nu}$, "paradoxical enhancement" (Kaufman et al., 1983), $F_i > F_j$ because $\bar{\nu}_i > \bar{\nu}_j$, caused by the flow bringing completely magnetized ensembles of nuclei into the receiver coil, can happen only when the magnetization already in the coil is not given a chance to relax completely between successive pulses: i.e., F_i, $F_j \ll 1$ because $t_r/T_1 \ll 5$. This is certainly not the case here since our value of t_r for the ^{23}Na spectra (0.256 s, the acquisition time) is more than five times the largest measured value of T_1 (0.035 s for the extracellular resonance). Thus, in our case increased $\bar{\nu}$ could only reduce F. Therefore, we must concern ourselves with the issue of whether the value of F is decreased more in one compartment than another because of different flows or within a given compartment because of a change in flow after an intervention. The values of $\bar{\nu}_j$ are certainly different (Gonzalez F., and J. B. Bassingthwaighte, manuscript in preparation; Singer, 1981). However, in our experiments, the values of T_1, t_r, L (~2.0 cm), and a reasonable value of d (~5 cm) are such that the value of $\bar{\nu}$ would have to approach 100 cm/s in order for F_{90} to be significantly decreased (Eq. 2). The value of $\bar{\nu}$ for the perfusing medium leaving the cannula is ~40 cm/s. This would be only lowered in the various cardiac compartments. Thus, there is little doubt that F_{90} is near one for all compartments and at all times.

We have advocated a rigorous non-NMR analytical determination of the amount of Na^+ or K^+, $n_{non\text{-}NMR}$, in any given compartment (Pike et al., 1984). Then, the visibility factor, v, can have a simple, empirical definition: $v \equiv$

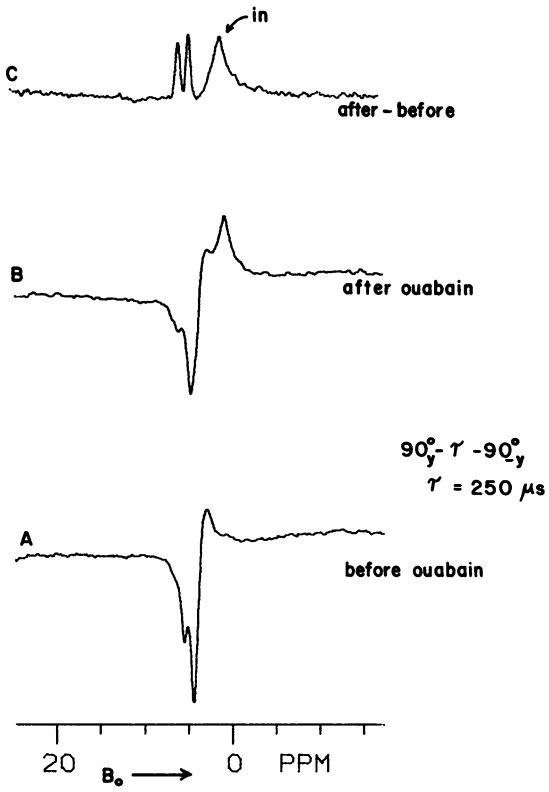

FIGURE 8 ^{23}Na jump-return NMR spectra (95.2 MHz, 8.5 T) of an isovolumic Langendorff-perfused beating rat heart. Before the acquisition of these spectra, the heart had been perfused for 31 min with the standard medium. The heart rate was 280 beats min^{-1}, the systolic/diastolic pressures 155/0, and the coronary flow 24 ml min^{-1}. Spectrum *A* was obtained at the end of a subsequent 43 min of perfusion with a solution containing 10 mM Dy(TTHA)$^{3-}$ and during which the pressures were 100/−5. Spectrum *B* was obtained after 27 min of a still further 47-min perfusion with a solution containing 10 mM Dy(TTHA)$^{3-}$ and 1 mM ouabain and during which the pressure was 50 mmHg. During all of these perfusions, the heart was bathed with a flowing solution containing 10 mM Dy(TTHA)$^{3-}$. There was also 10 mM Dy(TTHA)$^{3-}$ in the left ventricular balloon. For each jump-return spectrum, the delay time, τ, was 250 μs. Spectrum *C* is spectrum *B* − spectrum A. Its vertical scale has been expanded.

$n_{NMR}/n_{non\text{-}NMR}$ where n_{NMR} is the best analytical determination of the amount of Na^+ or K^+, present in any given compartment, possible under high-resolution NMR conditions. The value of ν can be <1 in a high resolution experiment due to quadrupolar effects (residual splitting and/or relaxation) and/or nonquadrupolar relaxation effects (Civan, 1983; Civan and Shporer, 1978; Berendsen and Edzes, 1973). Both of the ^{23}Na and ^{39}K nuclei are quadrupolar ($I = 3/2$). In a compartmentalized system as complex as the heart, ν could in principle take any value from 0 to 1. In simpler systems, a wide range of conditions yield the particular value of 0.4 (Civan, 1983; Civan and Shporer, 1978; Berendsen and Edzes, 1973). In other high-resolution studies, we have found that $\nu = 1$ in blood plasma (Pike et al., 1984) and in yeast suspension medium (Höfeler et al., 1984; Höfeler, H., J. A. Balschi, D. Jensen, M. Pike, V. P. Cirillo, J. Delayre, C. S. Singer, and E. T. Fossel, manuscript in preparation). The value of ν is also one in the cytoplasm of the human erythrocyte (Yeh et al., 1973; Pike et al., 1984; Pettegrew et al., 1984) but is <1 for intracellular Na^+ in yeast (Balschi et al., 1982; Ogino et al., 1983; Höfeler et al.). Gupta and co-workers published a ^{23}Na NMR study of packed human erythrocytes in which they report a $\nu < 1$ for Na_i (Gupta et al., 1982). However, since no shift reagent was used, the results could be interpreted as showing $\nu < 1$ for Na_o. Since we cannot conduct rigorous non-NMR analyses on the perfused, beating heart, we will leave the values of ν as variable.

Having appraised the values of all of the other factors of Eq. 1, we are in a position to predict the relative peak areas expected for the various compartments. We have done this in Table I for the isovolumic Langendorff heart for comparison with the ^{23}Na results and for the working heart for comparison with the ^{39}K results. In the calculations for Table I, we assumed that the Na^+/K^+ pump was not inhibited and that the shift reagent had reached steady state in the interstitial space. The latter allows us to combine the volumes of the chamber, vascular, and interstitial spaces into a single extracellular volume (V_o). We also assumed all values of F and ν to be one. Discrepancies between calculated and experimental results could be attributed to either or both of the latter. However, we have cited strong arguments above that the F values are always one.

We have used the curve resolution subroutine to determine that the area of the very small peak at ~0.8 ppm in sensitivity-enhanced ^{23}Na spectra of hearts with uninhibited Na^+/K^+ pumps (example/20.3 min, Fig. 2) is <1% of the total area (this is near our limit of detection for lines of similar widths and includes a correction for the bath signal seen in the spectrum obtained at time zero). This means that if c is truly ≥ 10 mM for Na_i, $\nu \leq 0.2$ (all the other uncertainties can make ν only smaller). If the broadening that causes the invisibility is of a heterogeneous nature (say, susceptibility effects related to the gross morphology of the heart), then less can be learned about the nature and level of Na_i. However, the reasonably sharp Na_i peak in the Na^+-loaded cells (Figs. 5–10) argues against this interpretation. If the broadening is homogeneous, a value of <0.4 for ν would imply that, at least in some compartment, more than quadrupolar effects are involved. The $m_I = +1/2$ to $m_I = -1/2$ transition of ^{23}Na (accounting for 40% of the intensity) is nonquadrupolar to first approximation. It may be that intracellular Na^+ is rather completely bound in the healthy heart cell. There has been speculation that it is "compartmentalized or sequestered" in the subcellular organelles of rabbit heart cells (Sperelakis, 1979; Lee and Fozzard, 1975). In the Results, we reported a value of $a_i/(a_i + a_o)$ of 0.64 for the ^{39}K spectrum in Fig. 4 *C*. Comparing this with the value predicted in Table I, we obtain $\nu_i = 0.09$ if we retain $\nu_o = 1$. Thus, for K_i, $\nu_i \leq 0.09$. It may be of qualitative (but only coincidental quantitative) significance that an earlier study determined the

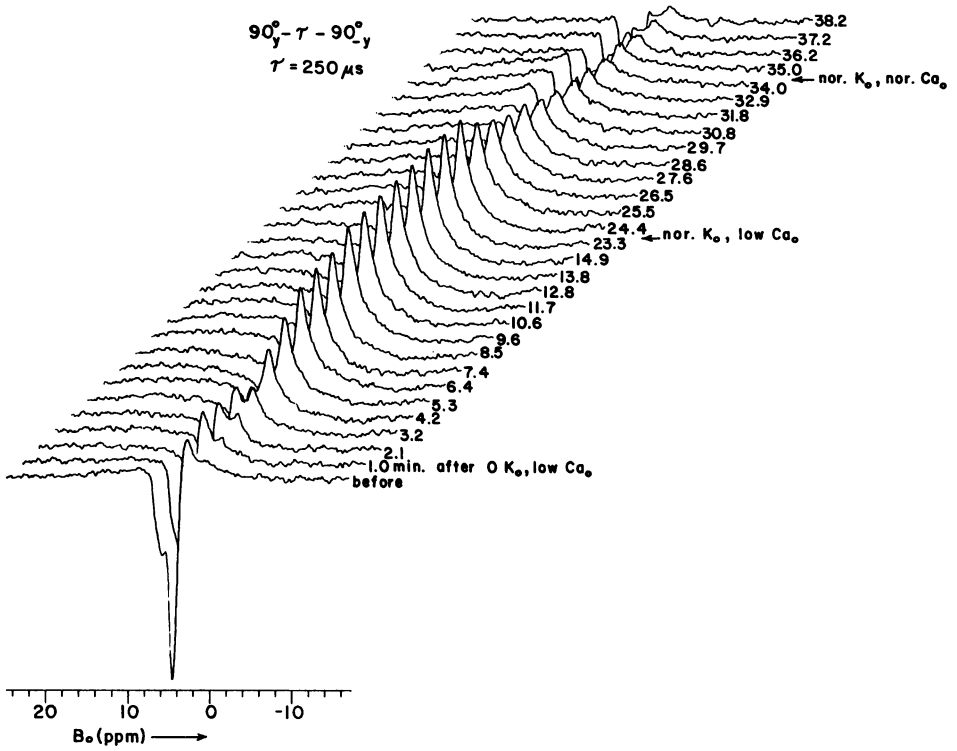

FIGURE 9 ^{23}Na jump-return NMR spectra (95.2 MHz, 8.5 T) of an isovolumic Langendorff-perfused beating rat heart. Before the acquisition of these spectra, the heart had been perfused for 24 min with the standard medium. The heart rate was 290 beats min^{-1}, the systolic/diastolic pressures 150/20 mmHg, and the coronary flow 23 ml min^{-1}. The first spectrum was obtained at the end of a subsequent 27 min of perfusion with a solution containing 10 mM $Dy(TTHA)^{3-}$ and during which the heart rate was 250 beats min^{-1}, the pressures 150/5, and the coronary flow 22 ml min^{-1}. Subsequent spectra are labeled with the times (midpoints of acquisitions) elapsed since a perfusion medium containing 10 mM $Dy(TTHA)^{3-}$, no K^+, and only 230 μM Ca^{2+} entered the heart. During the 23 min of this perfusion, the pressure was 100 mmHg. At 23.5 min, a new perfusion medium containing 10 mM $Dy(TTHA)^{3-}$, normal K^+ (5.9 mM) but still only 230 μM Ca^{2+} entered the heart. The subsequent pressure was 20 mmHg and the coronary flow was 18 ml min^{-1}. At 34.7 min, a new perfusion medium containing 10 mM $Dy(TTHA)^{3-}$ and normal K^+ and Ca^{2+} entered the heart. The subsequent heart rate was 230 beats min^{-1}, the pressures were 15/0 mmHg, and the coronary flow was 16 ml min^{-1}. During all of these perfusions, the heart was bathed with a flowing solution containing 10 mM $Dy(TTHA)^{3-}$. For each jump-return spectrum, the delay time, τ, was 250 μs and the number of transients was 128.

intracellular activity coefficients of Na^+ and K^+ in rabbit ventricular papillary muscles to be 0.175 and 0.612, respectively (Lee and Fozzard, 1975).

When excess Na^+ is allowed to accumulate inside the heart cells, either by the action of ouabain (Figs. 5, 6, 7, and 8) or by perfusing the heart with zero K_o (Figs. 9 and 10), a peak rises near the unshifted position. A plot of the area under the intracellular peak produced in jump-return difference spectra (Fig. 8) as a function of time elapsed after ouabain enters the heart is shown in Fig. 11. Similarly, the normalized areas of the intracellular peaks of jump-return difference spectra (Fig. 10) are plotted as a function of time in Fig. 12 to show the effect of temporarily lowering K_o to zero for the heart of Fig. 10. The time courses seen in Figs. 11 and 12 are very similar to those reported for the changes in activity of Na_i (measured by microelectrodes) in cardiac Purkinje fibers after analogous interventions (Deitmer and Ellis, 1978). We have no way of knowing whether the value of ν_i for ^{23}Na rises as excess Na^+ enters the cell. Because of this and other possible sources of error in the area measurements, we have refrained from reporting quantitative fluxes and reserve judgment on doing so until after further experimentation.

The transient changes in the lineshape of the shifted peak during shift reagent wash-in can be quite complex and interesting (Fig. 2). These vary somewhat from heart to heart and are probably impossible to explain completely. Under the conditions of our experiment, the main determinants of the magnitude of the shift are the ratio of shift reagent to Na^+ and the ratio of Ca^{2+} (and/or Mg^{2+}) to shift reagent (Chu et al., 1984). It seems likely that since the Na^+, Ca^{2+}, and Mg^{2+} concentrations in the vascular and interstitial spaces are constant, we are observing shift reagent entry into various capillary beds and crossing capillary walls into various interstitial spaces. It is likely that one is transiently discriminating portions of the vasculature and the interstitial spaces as shift reagent washes into the heart in a discrete fashion, both spatially and temporally (Little and Bassingthwaighte, 1983). In another context, we have discussed the theoretical bases for the effects of such discreteness on NMR lineshapes (Ting et al., 1981). Another aspect is the probable manifestation

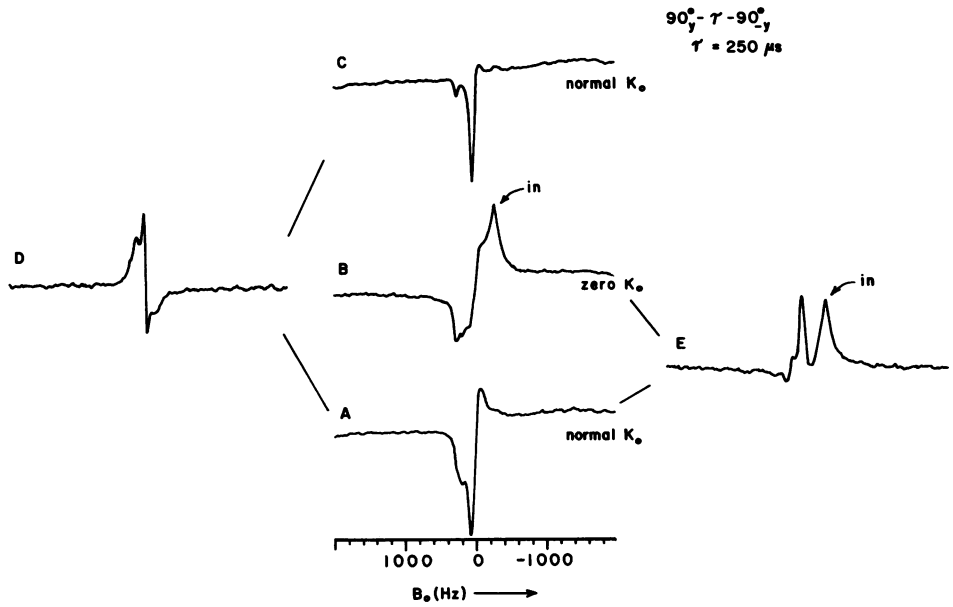

FIGURE 10 Selected spectra from the experiment of Fig. 9. Spectrum *A* is the first spectrum of Fig. 9. Spectrum *B* is that obtained at 23.3 min. Spectrum *C* was obtained at 41.4 minutes (not shown in Fig. 9). Spectrum *D* is spectrum *C* − spectrum *A*. Spectrum *E* is spectrum *B* − spectrum *A*. The vertical scales of spectra *D* and *E* have been expanded.

of bulk magnetic susceptibility shifts as the paramagnetic perfusing solution enters the various labyrinthine aspects of the cardiac compartments, the geometry of which must differ somewhat among hearts. Such shifts are highly geometry dependent (Becker, 1980). However, from the results above, one might expect such shifts to be small.

The transient changes in the lineshape of the shifted peak after inhibition of the Na^+/K^+ ATPase by ouabain (Figs. 5–7) are also interesting. Since the shift reagent ostensibly remains at steady state levels throughout the heart, the mechanism for these changes must be different than those offered in the preceding paragraph. A simple explanation is the occurrence of transient decreases in the Na^+ concentrations of various interstitial spaces as Na^+ enters cells. However, one might expect compensating increases in K^+ concentrations and these would tend to keep the shifts unchanged. Another intriguing possibility is an effect caused by the Na^+/Ca^{2+} exchanger in the sarcolemmal membrane. When the Na_i level rises, increased Ca^{2+} influx and/or reduced Ca^{2+} efflux (Chapman, 1983; Mullins, 1981; Sperelakis, 1979; Reuter and Seitz, 1968) could cause a transient drop in the level of Ca^{2+} in the interstitium until it could be replenished from the vascular network. Since Ca^{2+} competes with Na^+ for $Dy(TTHA)^{3-}$, it reduces the observed shift of the $^{23}Na^+$ resonance (Chu et al., 1984). Thus transient, and variable, reductions in the Na^+ and/or Ca^{2+} levels in the interstitial

TABLE I
ESTIMATION OF PEAK INTENSITIES

c_i(Na) assumed	$a_i/(a_i + a_0)$	
	Na^{+}*	K^{+}‡
10 mM	0.06	0.95
30 mM	0.17	0.95

*Assumes c_0 = 145 mM, V_i = 0.54, V_0 = 0.56 (0.10 [right ventricle] + 0.19 + 0.27; isovolumic Langendorff heart), $F_i = F_0 = \nu_i = \nu_0 = 1$.
‡Assumes c_i = 150 mM, c_0 = 5.9 mM, V_i = 0.54, V_0 = 0.66 (0.20 [ventricles] + 0.19 + 0.27; working heart), $F_i = F_0 = \nu_i = \nu_0 = 1$.

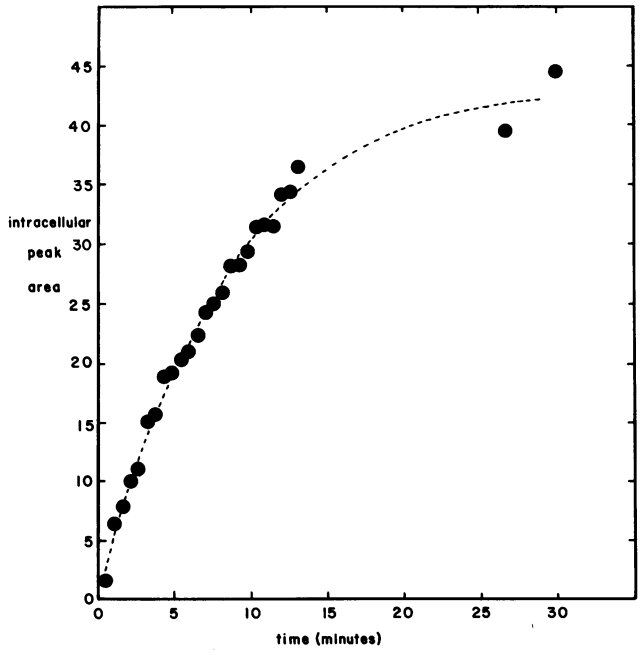

FIGURE 11 The time dependence of the area of the intracellular peaks (in arbitrary units) in jump-return difference spectra obtained for the experiment of Fig. 8. The dashed line is intended only to guide the eye.

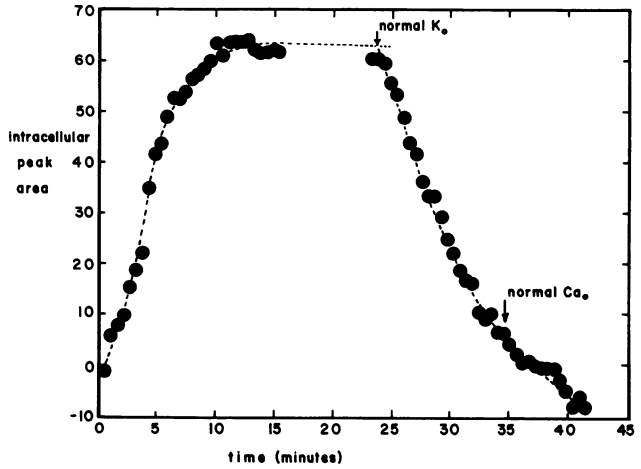

FIGURE 12 The time dependence of the area of the intracellular peak in jump-return difference spectra obtained for the experiment of Figs. 9 and 10. The arbitrary area unit is proportional to the ratio of the area of the intracellular peak in a jump-return difference spectrum to the area under the total heart ^{23}Na resonance in a normal spectrum of the same heart. The dashed line is intended only to guide the eye.

spaces could give rise to the transient increases in downfield shift observed for portions of the shifted resonance after introduction of ouabain (Figs. 5–7). Support for a Ca^{2+} contribution comes from one experiment in which we did not observe such large transient shifts when ouabain was administered after the introduction of 7 μM verapamil, a slow Ca^{2+} channel blocker, into the perfusion medium.

The higher level of Ca_i expected after zero K_o is also the reason for maintaining the perfusing medium at low Ca^{2+} for a brief period after returning K_o to normal. This is so that the Na^+/K^+ ATPase can return the Na_i to normal before Ca_o is restored to the normal value, thus avoiding Ca_i overload.

The decrease in area of the shifted resonance after introduction of ouabain (Figs. 5 and 6) also deserves comment. This decrease at steady state averaged 21% (with a large variance) for several hearts and is reflected in a negative-going shifted peak in simple difference spectra and the positive-going shifted peaks in the difference jump-return spectrum of Fig. 8 (because of the phase reversal of the residual shifted peaks caused by the pulse sequence). This phenomenon may result from a decrease in the value of V for the interstitial space. This could be caused by the cell swelling known to occur in cells with high Na_i levels after sodium-pump inhibition (MacKnight and Leaf, 1977; Cooke and MacKnight, 1984).

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