

## <sup>31</sup>P NMR spectroscopy of rat organs, *in situ*, using chronically implanted radiofrequency coils

(kidney/liver/heart/fructose/gated NMR)

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**ABSTRACT** A technique for making <sup>31</sup>P NMR spectroscopic measurements in rat kidney, heart, and liver *in vivo* is presented. Two-turn solenoid coils were surgically implanted around the organ sufficiently in advance of NMR experiments to allow recovery of the animal. These chronically implanted coils allowed acquisition of high-resolution spectra at 40.5 and 97.3 MHz. No resolution improvement occurred at the higher field. Spectra were stable for up to 24 hr, during which time a variety of experiments could be performed. By accumulating spectra at 10-min intervals, the effects of intraperitoneal fructose injections were monitored; in kidney and liver, a rapid increase in sugar phosphates at the expense of P<sub>i</sub> and ATP resulted. Fructose had no effect on heart metabolite levels. Spectra from the heart *in vivo* were obtained at systole and diastole by gating the spectrometer to the aortic pressure wave; no differences in phosphate metabolites were detected. Finally, saturation transfer techniques were used to monitor the rate of ATP synthesis in the kidney. The unidirectional rate constant for the conversion of P<sub>i</sub> to ATP was  $0.12 \pm 0.03 \text{ sec}^{-1}$ .

Recently, there has been much interest in the use of NMR to obtain biochemical information from animal tissue *in situ* (1-9). A major obstacle to such endeavors has been distinguishing signals due to the tissue of interest from those of nearby tissue. Organs lying close to the surface of animals have been studied by using surface coils (5-8). The addition of static-field focusing to define a small region of homogeneity (6) has the potential to study deeper lying organs. Other approaches have relied on acute surgical intervention to position an organ in the NMR receiver coil, concurrently removing it from surrounding tissue (2, 3, 9). These surgical maneuvers may have adverse physiological consequences and limit the viability of the organ or animal during the course of the-NMR measurements.

To circumvent some of these problems, a technique for implanting rf coils around rat kidney, liver, and heart several days prior to NMR experiments has been developed (10-12). These chronically implanted coils allow <sup>31</sup>P NMR spectra to be obtained from organs under homeostatic conditions with any spectrometer that has a magnet large enough to accommodate the animal.

### MATERIALS AND METHODS

**Surgical Procedures.** Sprague-Dawley rats (180-220 g) maintained on a standard diet were used throughout. Animals were anesthetized with an intraperitoneal injection of a mixture of Ketalar (87 mg/kg of body weight; Parke, Davis, Morris Plains, NJ) and Rompun (3 mg/kg of body weight; Cutter,

Shawnee, KS). The left kidney was exposed by a flank incision and the coil was placed around the organ. To separate the surrounding muscle from the coil, a thin layer of a biologically inert material, Gortex (Gore, Sunnyvale, CA), was placed between the coil and the muscle. The coil was held in place by tightly suturing the muscle and skin around the coil leads during closure. Liver coils were placed between the hepatic lobes through an abdominal incision. No Gortex was needed as the coil was surrounded on all sides by liver.

To implant heart coils, the animal was anesthetized and maintained on positive pressure ventilation via tracheal intubation through the mouth. The diaphragm was exposed through a midabdominal incision and then opened with an arc-shaped incision. The coil was placed around the heart and the diaphragm was then closed around the leads using sutures and liquid adhesive (Loctite Superbond 495; Loctite, Newington, CT). The coil leads were passed to the exterior through the abdominal skin. A soft polyethylene tube was left in the thoracic cavity; negative suction was applied for 2-6 hr after surgery. Prophylactic antibiotics (PR) were administered. Recovery from all surgery was monitored by daily measurement of weight.

For NMR measurements, the rat was continually anesthetized with a mixture of Ketalar (43.5 mg/hr per kg of body weight) and Rompun (1.5 mg/hr per kg of body weight) through an intraperitoneal line (PE-50, Clay Adams). A venous line, for continual infusion of saline, and an arterial line, to monitor blood pressure and heart rate, were inserted. The animal was placed on a holder, the coil leads were soldered to the rest of the tuning circuit, and the circuit was tuned to the appropriate frequency. The holder was then placed in a shielded casing and positioned so that the organ of interest was centered in the static magnetic field ( $B_0$  field). At the end of an experiment, the organ was preserved for histological examination.

**Coil Design.** Coils were made from 22-gauge copper wire insulated with polyethylene tubing (PE-100, Clay Adams). To minimize loss in sensitivity associated with the wire leads, which ran from the coil to the tuning circuit outside the rat, fixed value capacitors (American Technical Ceramics, Huntington Station, NY) were attached close to the coil to partially tune and match the circuit (13). The capacitors were insulated with silicone sealant and covered with Teflon tape. In some cases, a capillary containing methylenediphosphonic acid (MDPA, Sigma) buffered at pH 8.9 was attached to the coil during surgery to provide a chemical shift and integration standard.

**NMR Measurements.** <sup>31</sup>P NMR was measured at 40.5 MHz on a Varian XL-100 with a MONA accessory (Nicolet, Fremont, CA) and at 97.3 MHz on a home-built spectrometer using a su-

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Abbreviations: MDP, methylenediphosphonic acid; 2,3-DPG, 2,3-diphosphoglycerate; P-Cr, phosphocreatine;  $B_0$  field, static magnetic field.

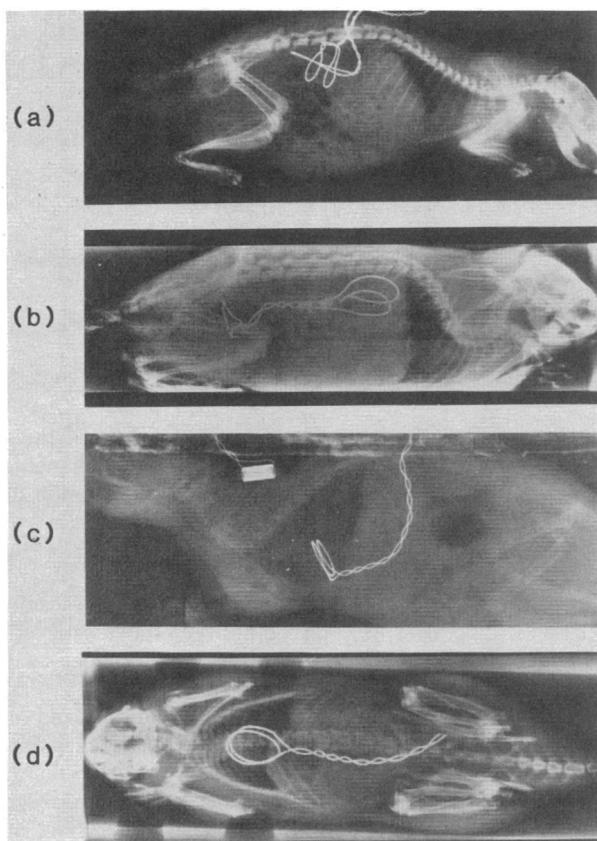


FIG. 1. X-rays of rats 2 days after coil implantation showing orientation of the coils. In experiments performed at 40.5 MHz (a and c), the  $B_0$  field runs perpendicular to the plane of the page; at 97.3 MHz (b and d), the  $B_0$  field runs parallel to the long axis of the rat. (a) Coils implanted around kidney. The two-turn coil was used to detect  $^{31}\text{P}$  and the single-turn coil for  $^1\text{H}$  to shim and lock the  $B_0$  field. (b) Kidney coil. (c) Heart coil. The coil external to the rat contained a capillary of  $^2\text{H}_2\text{O}$  for locking the field. (d) Heart coil.

perconducting magnet with a 7.6-cm-diameter bore (Cryomagnets Systems, Indianapolis, IN) and a Nicolet 1180/293B data system. The XL-100 instrument was locked to protons detected from the organ by using a second, crossed coil tuned for protons. The fixed-frequency  $^2\text{H}$  lock channel of the XL-100 was converted to observe protons by using a mixer placed between the  $^2\text{H}$  preamplifier and the probe. An additional frequency synthesizer was used to mix the  $^2\text{H}$  pulse frequency up for excitation and back down for detection. Because the proton signal is so strong, the loss in sensitivity associated with this scheme is tolerable. No lock was used with the superconducting magnet. The proton signal arising from water in the organ was used to shim, typically to a  $^1\text{H}$  line width of 45–70 Hz, prior to acquiring  $^{31}\text{P}$  spectra.

To quantitate peak areas and chemical shifts, the Nicolet simulation routine NTCCAP was used. Line widths, heights, and peak positions were varied until a minimum rms-error fit was obtained. No resolution enhancement techniques were used to remove broad components associated with any of the spectra.

For  $T_1$  measurements, the recovery of the spectrum was monitored at a sequence of times following a saturating burst of 5–10  $90^\circ$  pulses, alternated with 10-msec homospoil pulses.  $T_1$  values of MDPA in a capillary measured in a rat and in a conventional NMR probe were found to agree within 10%, confirming the accuracy of such measurements.

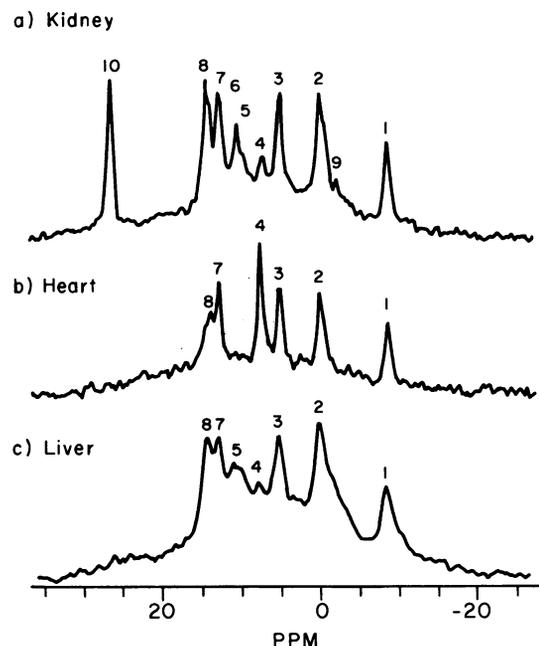


FIG. 2. Spectra obtained from kidney (a), heart (b), and liver (c) by using chronically implanted coils. Spectra are the sum of 128 acquisitions using a 30- $\mu\text{sec}$  pulse ( $75^\circ$ ) and a 10-sec recycle time. A 30-Hz exponential filter was applied to the free induction decay prior to Fourier transformation. Chemical shifts were referenced to the ATP  $\alpha$ -phosphate peak, which was set equal to 0 ppm. Peaks: 1, ATP  $\beta$ -phosphate; 2, ATP  $\alpha$ -phosphate/NAD(H); 3, ATP  $\gamma$ -phosphate; 4, *P*-Cr; 5, phosphodiester; 6, urine  $\text{P}_i$ ; 7,  $\text{P}_i$ ; 8, phosphomonoester; 9, unknown; 10, MDPA.

## RESULTS AND DISCUSSION

**Coil Implantation.** Surgical implantation of NMR rf coils is an invasive procedure, the effects of which were largely reversible when the animals were allowed to recuperate for several days. All rats survived kidney and liver surgery and were gaining weight within 2 days. The survival rate from heart surgery was approximately 80%, with weight gain beginning within 4 days. Histological examination showed that minor surface fibrosis of kidney, liver, and heart occurred in tissue that touched the coil.

X-rays (Fig. 1) show that both kidney and heart coils maintained their proper orientation perpendicular to the  $B_0$  fields, even though the rats were allowed to move about freely. Coils placed in the liver also maintained proper orientation.

**Quantitative Analysis of Spectra.**  $^{31}\text{P}$  NMR spectra of kidney, heart, and liver obtained at 97.3 MHz (Fig. 2) indicate the relative concentrations (Table 1) of observed metabolites. There was no significant difference in the intensities of the ATP  $\beta$ - and  $\gamma$ -phosphate peaks in any of the organs, indicating that free ADP concentrations were <3% of ATP concentrations.

Peaks observed *in vivo* and their chemical shifts are in agreement with those detected in perfused organs (3, 14–18), although there are some differences. In the phosphodiester region of the kidney spectrum, a previously unreported peak (Fig. 2a, peak 6) is seen along with contributions from a previously reported (7) phosphodiester peak (peak 5). This new peak changed in intensity and chemical shift concomitantly with changes in urine pH (unpublished data), suggesting that it is due to urine  $\text{P}_i$  contained in the kidney pelvis. In the *in vivo* kidney, an unidentified peak is detected (Fig. 2a, peak 9), where nucleotide sugars, such as UDP-glucose, are expected to resonate.

Phosphocreatine (*P*-Cr) is detected in neither the perfused

Table 1. Relative intensities and chemical shifts of metabolites

	ATP phosphate			P-Cr	PDE	P <sub>i</sub>	SP
	β	α [+NAD(H)]	γ				
Kidney	100	208 ± 26*	99 ± 2	30 ± 16	31 ± 25	103 ± 26	150 ± 19
Heart	100	182 ± 15	103 ± 3	133 ± 15	3 ± 5	116 ± 8	107 ± 37
Liver	100	191 ± 14	101 ± 1	14 ± 9	90 ± 84	57 ± 19	106 ± 9
Shift†	-8.64	0	5.15	7.55	10.55	12.76	14.22

Intensities are in arbitrary units relative to ATP β-phosphate = 100. Results are mean ± SD for *n* experiments; *n* = 10 for kidney; *n* = 6 for heart; *n* = 2 for liver. PDE, phosphodiester; SP, sugar phosphates.

\*Includes an unknown peak at -2.31 ppm.

†Shifts are in ppm relative to ATP α-phosphate = 0.

liver nor the kidney (3, 14, 17, 18), so the amount of *P*-Cr seen *in vivo* indicates the contribution of surrounding muscle. In rat skeletal muscle, the *P*-Cr/ATP ratio is 4:1 and the *P*-Cr/P<sub>i</sub> ratio is 15:1 (5). The *P*-Cr/ATP ratio detected in kidney was ≈1:3.3 and that in liver was less (Table 1). If all the *P*-Cr were coming from muscle, then other muscle metabolites, such as P<sub>i</sub> and ATP, would make a <10% contribution to the spectrum. To verify this, the renal artery was ligated; the level of ATP, monitored by the height of the ATP β-phosphate peak, dropped to zero within 15 min, with a complementary increase in P<sub>i</sub>. Were surrounding tissue contributing to the spectrum, one would expect to observe some residual ATP signal. Furthermore, when the kidney was removed and replaced with a phantom containing only MDPA, no narrow metabolite peaks were detected. A broad feature that persisted is suggested to arise from metabolites in surrounding tissue residing in inhomogeneous portions of the B<sub>0</sub> field and probably contributes to all *in vivo* spectra.

Besides the tissue of interest, blood circulating through the organ can contribute to the spectrum. In particular, the phosphomonoester-P<sub>i</sub> region of the *in vivo* heart spectrum has a higher intensity than that observed in the working heart perfused with phosphate-free medium (15). This suggests either that there is a significant contribution to the spectrum from 2,3-diphosphoglycerate (2,3-DPG) and P<sub>i</sub> in blood within the heart

chamber or that changes in cardiac metabolism account for the differences. However, even if all the phosphomonoester-P<sub>i</sub> intensity in the heart spectrum were due to blood, the concentration of ATP occurring in rat blood is too small to make a significant contribution (unpublished data). The effect of blood on the liver and kidney spectra is harder to estimate because the *in vivo* spectra closely resemble those obtained from the perfused organ. It is estimated that blood and extracellular fluid comprise ≈15% of the wet weight of liver and kidney (19). From the concentration of 2,3-DPG of rat blood (20) and the 2,3-DPG/P<sub>i</sub> ratio, determined from NMR of whole rat blood (unpublished data), it can be estimated that blood contributes ≈25% of the phosphomonoester-P<sub>i</sub> intensity in both kidney and liver. This implies that other metabolites in blood make negligible contributions to the organ spectra.

**Magnetic Field Effects.** To determine the effects of field strength on resonance line widths from organs *in vivo*, spectra were obtained at 40.5 and 97.3 MHz (Fig. 3). Line widths increased roughly in proportion to the field strength, explaining why no improvement in resolution was obtained at the higher field. This result is consistent with results obtained on cell suspensions (21). A quantitative description of line-width dependence on field is difficult with such complicated systems because many mechanisms may contribute to the line broadening—

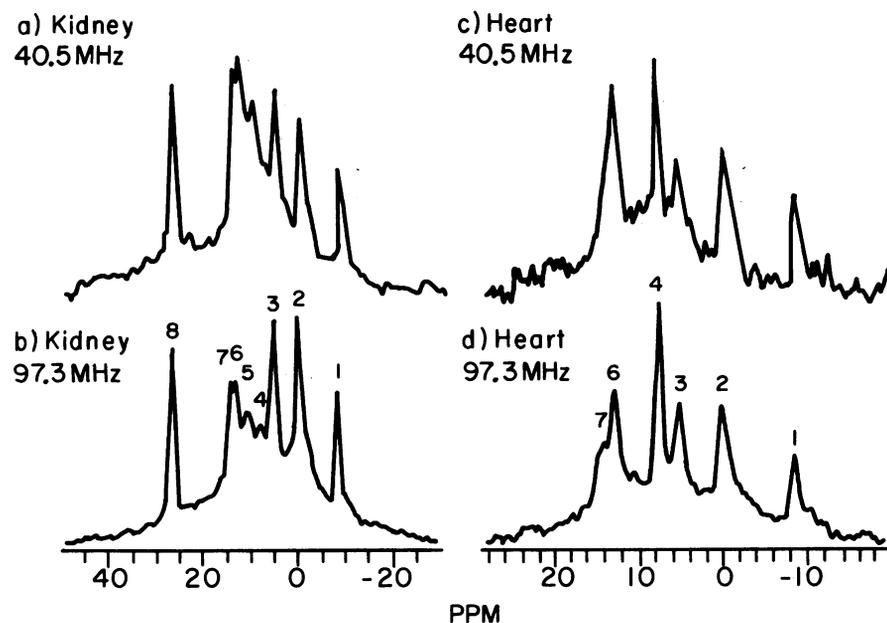


FIG. 3. Comparison of kidney (a and b) and heart (c and d) <sup>31</sup>P NMR spectra at 40.5 MHz (a and c) and 97.3 MHz (b and d). Acquisition parameters were 45° pulse, 130-msec recycle time, 30-min total acquisition time, with a 15-Hz filter (a), 45° pulse, 130-msec recycle time, 8-min total acquisition time, and a 30-Hz filter (b), 60° pulse, 3-sec recycle time, 30-min total acquisition time, and a 15-Hz filter (c), and 90° pulse, 10-sec recycle time, 20-min total acquisition time, and a 30-Hz filter (d). Peaks: 1, ATP β-phosphate; 2, ATP α-phosphate/NAD(H); 3, ATP γ-phosphate; 4, *P*-Cr; 5, urine P<sub>i</sub>/phosphodiester; 6, P<sub>i</sub>; 7, phosphomonoester; 8, MDPA.

e.g., chemical shift dispersion arising from pH heterogeneity, paramagnetic ions, and residual chemical shift anisotropy. In addition, micro- and macrostructural heterogeneity in an organ can lead to line broadening induced by geometrical effects or differences in susceptibilities or both.

**Stability of Spectra.** An advantage of *in vivo* experiments over studies of perfused organs is metabolic stability and integrity. With implanted coils, NMR spectra can be obtained continuously for 18–24 hr, with only a  $10 \pm 3\%$  variation in the intensity of the ATP  $\beta$ -phosphate peak in kidney or the P-Cr/ATP ratio in heart. This permits a wide variety of maneuvers and observations on a single animal. The lengths of experiments were limited by variability of animal response during anesthesia. Unanesthetized animals could be used to obtain excellent spectra but small movements were frequent and required continual reshimming or repositioning. The use of tranquilizers would minimize movements and eliminate the adverse effects of prolonged anesthesia.

**Effects of Fructose.** Previous work, using both freeze-clamp techniques (22–25) and NMR (4, 11, 26), has shown that fructose administration depletes ATP and  $P_i$  levels while increasing sugar phosphate levels in kidney and liver. Comparison of the time course of metabolite levels in both kidney and liver after an intraperitoneal injection of fructose is shown in Fig. 4. In both organs, levels of  $P_i$  decrease rapidly as sugar phosphate levels increase. The effect was much more pronounced in liver than in kidney, which is in agreement with freeze-clamp data (23, 25). The parallel changes in the ATP  $\beta$  and  $\gamma$  resonances indicate that ADP levels never become detectable. However, levels of ATP did fall, indicating that the net pool of adenylate decreases. The fact that ATP levels recovered slowly, relative

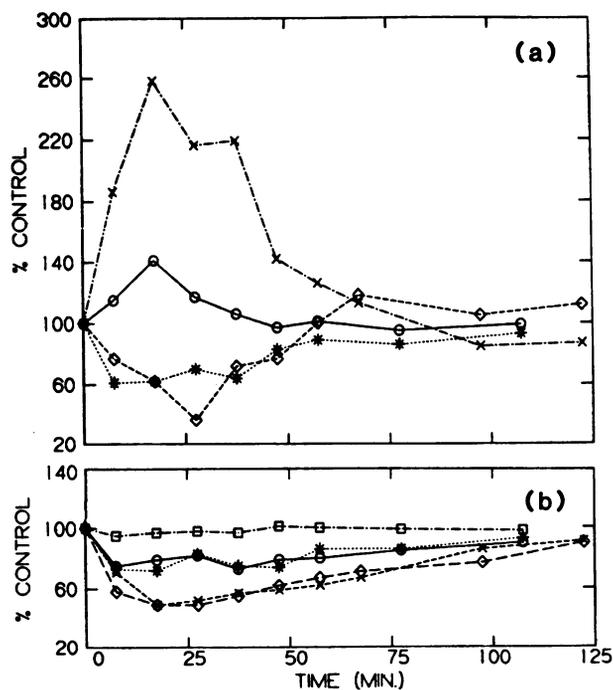


FIG. 4. An intraperitoneal injection of fructose ( $10 \mu\text{mol/g}$  of body weight) was administered and metabolite levels were monitored in kidney and liver. After obtaining three control spectra, acquisitions were averaged for 5 min every 10 min beginning 5 min after the fructose injection. Spectra were acquired at 97.3 MHz using two hundred and eighty-six  $90^\circ$  pulses, a 1-sec recycle time, and a 30-Hz filter. The following metabolites are shown. (a)  $\circ$ , Kidney sugar phosphate;  $*$ , kidney  $P_i$ ;  $\times$ , liver sugar phosphate;  $\diamond$ , liver  $P_i$ . (b)  $\circ$ , Kidney ATP  $\gamma$ -phosphate;  $*$ , kidney ATP  $\beta$ -phosphate;  $\times$ , liver  $\gamma$ -phosphate;  $\diamond$ , liver ATP  $\beta$ -phosphate;  $\square$ , MDPA in a capillary attached to the kidney coil.

to those of  $P_i$  and sugar phosphates, is consistent with the idea that adenine nucleotides must be resynthesized to replace the depleted pool (22, 25). Fructose loading did not alter the heart spectrum.

**NMR Gated to the Cardiac Cycle.** Using gated NMR, Fossel *et al.* (27) have found cyclical concentration changes of phosphate metabolites in the glucose-perfused working heart. To determine whether these cyclical changes occur *in vivo*, gated heart spectra were obtained (Fig. 5). Two time intervals were monitored: peak systole and a point 200 msec later, during diastole. The relative position of the diastolic point varied 10% with respect to peak systole because of variations in heart rate. There was no difference (Fig. 5e) between the spectra obtained at diastole (Fig. 5c) and at systole (Fig. 5d). The inconsistencies between the results obtained on the perfused heart (27) and the *in situ* heart may be due to the better nutrient supply available *in vivo*.

**Saturation Transfer.** Steady-state metabolite-turnover rates have been measured by magnetization-transfer techniques in isolated cell suspensions (28–30), in perfused organs (31–35), and in the *in vivo* brain, using a surface coil (36). Fig. 6 shows a  $^{31}\text{P}$  NMR saturation-transfer experiment performed using a coil implanted around the rat kidney. To quantitate exchange between  $P_i$  and ATP  $\gamma$ -phosphate, spectra with the ATP  $\gamma$ -phos-

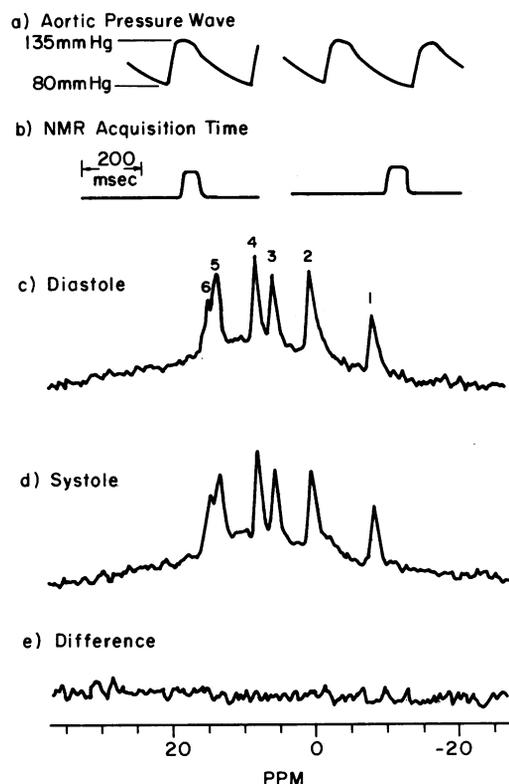


FIG. 5. Gated  $^{31}\text{P}$  NMR spectra obtained from the *in vivo* rat heart. (a) Aortic pressure wave obtained with a polyethylene line in the carotid artery and a Stentor pressure transducer. To ensure that the line introduced no significant time delay, the pressure wave was compared with the electrocardiogram in a parallel experiment outside the magnet. (b) NMR acquisition interval displayed on the same scale as the pressure wave, demonstrating the ability of the gating device to properly couple the pressure wave to the 293B pulse programmer. Spectra obtained at peak systole (d) and 200 msec later, diastole (c), were acquired at 97.3 MHz by alternating four acquisitions ( $45^\circ$  pulse, 2-sec delay, 30-Hz filter) at each interval until 480 scans at each point were obtained. (e) Diastole minus systole. Peaks: 1, ATP  $\beta$ -phosphate; 2, ATP  $\alpha$ -phosphate/NAD(H); 3, ATP  $\gamma$ -phosphate; 4, P-Cr; 5,  $P_i$ ; 6, phosphomonoester.

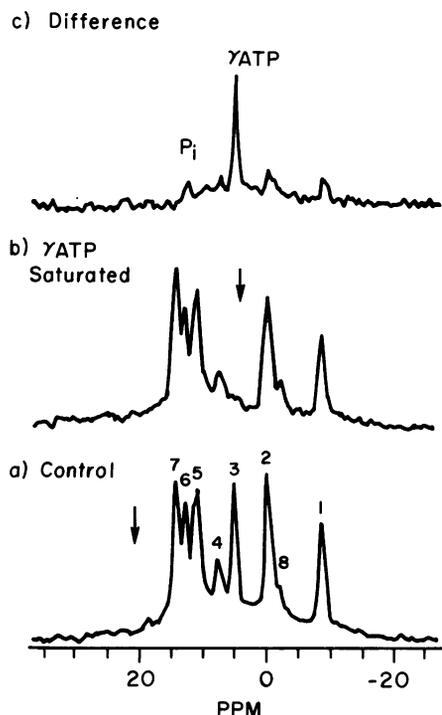


FIG. 6. <sup>31</sup>P NMR saturation-transfer measurement of the P<sub>i</sub> to ATP γ-phosphate exchange of *in vivo* kidney. (a) Control spectrum. (b) Spectrum obtained with the ATP γ-phosphate peak saturated. Arrows indicate positions of saturating pulses. (c) Difference spectrum indicating the decrease in P<sub>i</sub> magnetization when the ATP γ-phosphate peak was saturated. The work load of the left kidney was increased by removing the right kidney 5 days prior to NMR experiments and volume loading (4% of body weight) the rat with plasma just prior to NMR measurements. Spectra were obtained at 97.3 MHz by alternating 8 acquisitions with the saturating pulse at each frequency until 128 acquisitions had been obtained for each spectrum. A 90° pulse, 10-sec delay, during which the saturation pulse was on, and a 30-Hz filter were used. Peaks: 1, ATP β-phosphate; 2, ATP α-phosphate/NAD(H); 3, ATP γ-phosphate; 4, P-Cr; 5, urine P<sub>i</sub>/phosphodiester; 6, P<sub>i</sub>; 7, phosphomonoester; 8, unknown.

phate saturated (Fig. 6b) and a control (Fig. 6a) were obtained. The difference (Fig. 6c) shows the reduction in P<sub>i</sub> magnetization caused by exchange of observable P<sub>i</sub> magnetization for saturated ATP γ-phosphate. Using this information together with the T<sub>1</sub> of P<sub>i</sub> measured with the ATP γ-phosphate saturated (1.24 ± 0.16 sec), the unidirectional rate constant for the process P<sub>i</sub> to ATP was calculated (32) to be 0.12 ± 0.03 sec<sup>-1</sup>.

### CONCLUSIONS

Stable <sup>31</sup>P NMR spectra from the heart, kidney, and liver of laboratory rats can be obtained by using chronically implanted coils. Rats have been observed to tolerate the coils for up to 6 months, the longest time monitored, allowing the possibility of repeated study of a single animal. Signal-to-noise ratios obtained were comparable with those achieved with perfused organs (3, 14–18) making possible a wide variety of experiments. Future developments should permit simultaneous measurement of NMR spectra and physiological data. This technique can easily be extended to larger animals, other organs, and other nuclei.

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