PROTON DECOUPLED FLUORINE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN SITU

BRUCE A. BERKOWITZ AND JOSEPH J. H. ACKERMAN
Department of Chemistry, Washington University, St. Louis, Missouri 63130

ABSTRACT The efficacy of proton decoupling for enhancing the ^{19}F nuclear magnetic resonance (NMR) signal-to-noise ratio and spectral resolution in the intact subject is demonstrated. A geometrically orthogonal cross-coil antenna configuration (Helmholtz pair, surface coil) is employed to provide 40 dB of isolation between the ^{19}F observe and ^{1}H decouple frequencies of 188 and 200 MHz, respectively. Further isolation is achieved through the use of high-quality notch filters on both observation and decoupling channels. Application of ^{19}F-$\{^{1}H\}$ NMR spectroscopy to the study of 2-fluoro-2-deoxy-D-glucose metabolism in cerebral tissue in situ is presented. Significant improvements in sensitivity and resolution are obtained and result from both a collapse of the J_{FH} multiplet structure and a substantial positive nuclear Overhauser effect (NOE). To our knowledge, this is the first such demonstration of ^{1}H decoupling in conjunction with ^{19}F observation for study of the metabolism of a fluorinated compound in the living subject.

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy, coupled with surface coil technology (1), has become an important noninvasive approach to studying evolving metabolism in situ. Naturally occurring, nonradiative, spin ½ labels such as ^{1}H, ^{13}C, and ^{31}P have proven useful in elucidating metabolism in vivo. The ^{19}F nuclide offers many advantages as an exogenous spin ½ label including a large spectral dispersion (~500 ppm), high sensitivity (~0.8 that of ^{1}H), and a natural abundance of 100%. The negligible endogenous fluorine concentration eliminates spectral background interference in tissue and, thus, in principle, ^{19}F NMR analysis of the metabolism of a fluorine-labeled substrate in situ can be readily accomplished (2, 3).

A potential problem encountered when using a fluorine label for studying metabolism is scalar coupling interactions with neighboring protons. In the case of ^{19}F-^{1}H scalar couplings (J_{FH}) on the order of or greater than the susceptibility inhomogeneity broadened tissue resonance linewidths ($\Delta\upsilon_{1/2}$ tissue ~0.2–0.4 ppm), one can expect a marked decrease in the apparent frequency resolution (i.e., an increase in the observed linewidth) compared with the frequency resolution in the absence of the J_{FH} interaction. Under these circumstances (and in the absence of a negative nuclear Overhauser effect (NOE), vide infra), the narrower lines of the proton decoupled ^{19}F NMR spectrum should present a higher apparent signal-to-noise ratio than the proton coupled spectrum.

Correspondence should be addressed to Dr. Joseph J. H. Ackerman.

Proton decoupling is routinely used in ^{13}C NMR to collapse multiplet patterns produced by ^{13}C-^{1}H scalar coupling interactions and thereby simplify the spectrum; additional signal enhancement is achieved via the NOE. Because of the substantial difference between the radio frequencies (rf) of the observe and decouple channels in the ^{13}C-$\{^{1}H\}$ experiment, appropriate double resonance circuit design is straightforward and many isolation schemes have been reported and utilized to good effect. Unfortunately, unlike the ^{13}C-$\{^{1}H\}$ experiment the ^{19}F and ^{1}H nuclides are separated by only ~6% in frequency, making proton decoupling while observing fluorine a potentially difficult problem with regard to achieving adequate rf isolation between the observe and decouple channels while maintaining receiver efficiency.

In the case of low molecular weight, highly mobile fluorinated compounds, a theoretical increase in ^{19}F sensitivity of 53% through the ^{19}F-^{1}H NOE can be achieved upon ^{1}H decoupling (4) in addition to the signal-to-noise increase resulting from collapse of the J_{FH} multiplet structure. However, Hull and Sykes (5) have reported that less mobile fluorinated molecules can experience decreases in sensitivity through the (negative) NOE. Therefore, fluorinated compounds existing outside the extreme narrowing regime, and which might be found in vivo, may require the use of special gated decoupling techniques to avoid sensitivity losses due to adverse (i.e., negative) NOE effects.

Herein we demonstrate the resolution and signal-to-noise enhancements that can be obtained through the use of a geometrically orthogonal cross-coil antenna configuration for proton decoupling of fluorine metabolites in the intact functioning subject in situ. This orthogonal configu-

ration provides excellent frequency-independent rf isolation, optimizes the ^{19}F observation and ^{1}H decoupling antenna efficiency, and is easily implemented in a standard vertical wide-bore, high-field, high-resolution NMR spectrometer.

EXOGENOUS FLUORINATED METABOLIC SUBSTRATE

Powerful radiological imaging techniques employing ^{18}F labeled 2-fluoro-2-deoxy-D-glucose (2FDG) have been applied to map local cerebral glucose utilization rates in humans (6, 7). Due to near exclusive use of glucose as a cerebral energy source, this map of cerebral glucose utilization rates can, in principle, be interpreted as a map of regional cerebral activity (6, 7). However, recent controversy over the metabolic fate of deoxy-glucose analogues and, thus, their applicability as quantitative monitors of cerebral activity (8) has motivated the development of ^{19}F NMR techniques to delineate 2FDG metabolism in situ (9–12).

The sensitivity of the fluorine nuclide to the scalar coupling of neighboring protons is illustrated in the high resolution, proton coupled and decoupled ^{19}F NMR spectra of a mixture of 2FDG and its hexokinase phosphorylation product 2-fluoro-2-deoxy-D-glucose-6-phosphate (2FDG6P) in aqueous solution (Fig. 1). The proton decoupled ^{19}F spectrum of 2FDG and 2FDG6P contains four primary resonances reflecting glucose anomerization, namely the β and α forms (Fig. 1 *A*). Progressively increasing the mole fraction of 2FDG6P by stepping of the ATP concentration in a reaction mixture of 2FDG, KCl and/or $MgCl_2$, and hexokinase provided positive differentiation of those resonances due to 2FDG and 2FDG6P in the high resolution proton decoupled ^{19}F NMR spectrum. Relative to trifluoroacetate, which was used as an internal ^{19}F chemical shift reference and assigned to 0.00 ppm, the α, β anomers of 2FDG and 2FDG6P have shifts of -123.84, -123.68, -123.92, and -123.66 ppm, respectively (37°C, 120 mM ionic strength, pH = 7.1). These assignments are similar but somewhat in contrast to those reported elsewhere (9, 14). These shifts were found to be insensitive to ionic strength variations about the physiologic norm, ~120–180 mM. Furthermore, in comparing shifts from a solution containing a high concentration of monocation (90 mM KCl, 2 mM $MgCl_2$) with a solution containing a high dication concentration (0 mM KCl, 48 mM $MgCl_2$), no marked ^{19}F chemical shift perturbations

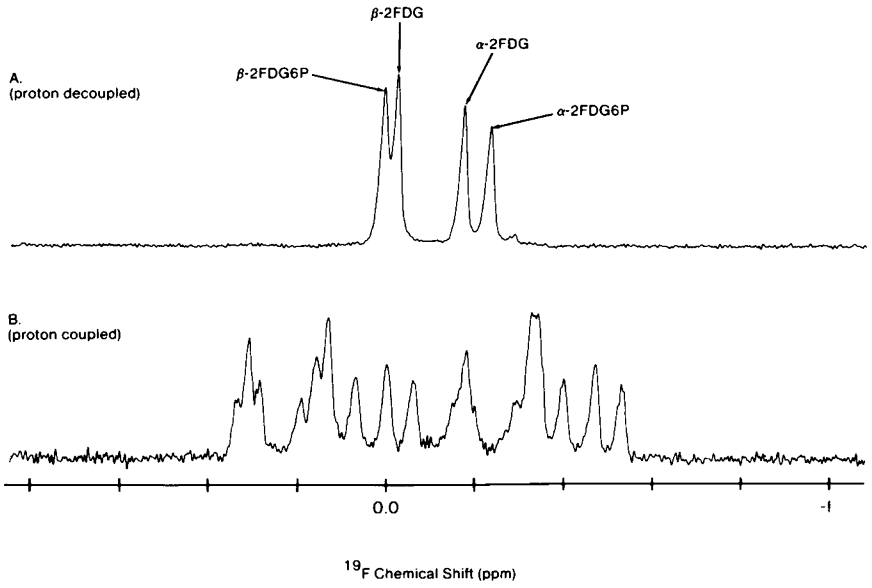

FIGURE 1 Fluorine NMR spectra of 2FDG (Behring Diagnostics, American Hoechst Corp., San Diego, CA) and its phosphorylated product 2FDG6P obtained under proton decoupled (*A*) and proton-coupled (*B*) conditions. For definitive identification of the anomer resonances of 2FDG and 2FDG6P, the composition of a solution of 2FDG and 2FDG6P was varied from 100% 2FDG to 100% 2FDG6P by a modification of the procedure of Bessell and Thomas (14). The solution composition (relative amounts of substrate 2FDG and product 2FDG6P) was varied in a stepwise manner by phoshorylation with yeast hexokinase (Sigma Chemical Co., St. Louis, MO) with the extent of reaction limited by added ATP. Ionic strength was maintained about the physiologic norm (~120–180 mM) with pH = 7.1 and temperature = 37°C; 20% D_2O was used as an internal lock and trifluoroacetate (1.4 mg/ml) was present as an internal ^{19}F chemical shift reference. The reaction was followed by monitoring the pH. The final pH was adjusted to 7.1 and an aliquot was taken for ^{19}F NMR analysis. Time domain data was acquired on a pulsed Fourier transform NMR spectrometer (model FX-100; JEOL USA, Analytical Instruments Div, Peabody, MA) operating at 93.67 MHz for ^{19}F detection. Spectra represent 4 h of data acquisition (2 K data points, 1 kHz band width, 1.5 s pulse repetition period). Fourier transformation was performed after zero filling to 16 K; no sensitivity enhancing digital filtering was employed. IUPAC chemical shift convention was followed (22) with the β-anomer of 2FDG6P (decoupled) arbitrarily assigned 0.0 ppm in this figure; actual assignment is -123.66 ppm relative to trifluoroacetate as an internal standard. Spectral assignments and multiplet patterns are more fully discussed in text. In (*A*) the small peak to lower frequency is unassigned. Abscissa divisions are in units of 0.2 ppm.

ascribable to cation interactions (with the electronegative fluorine or phosphate group) were detected.

The presence of J_{FH} interactions splits each anomer resonance into distinct multiplet patterns (Fig. 1 *B*), which have been analyzed previously (9, 13); the multiplet pattern due to J_{FH} of 2FDG6P is very similar to that described for 2FDG (9, 14). The largest coupling of ~50 Hz is due to the geminal proton on the C-2 carbon. The vicinal proton on the C-3 carbon of 2FDG and 2FDG6P provides a 15-Hz coupling that further splits each resonance. Finally, when the vicinal proton on the C-1 carbon is axial (i.e., when the glucose analogue is in the β-anomer form) a 2.5-Hz coupling is observed under high resolution conditions; if this vicinal proton is equatorial (α-anomer), then it does not couple with the fluorine on the C-2 carbon to any significant extent. Based on the presence and absence of the J_{FH} interaction with the vicinal proton at C-1, the two proton decoupled resonances at high frequency in Fig. 1 *A*, are identified as the β-anomers. The 50 Hz geminal J_{FH} interaction is clearly on the order of the tissue resonance linewidth in situ and so proton decoupling can be expected to significantly enhance the observed frequency resolution in the ^{19}F NMR spectrum of 2FDG and 2FDG6P in tissue. In the absence of a negative NOE, a signal-to-noise enhancement should result from this increased resolution.

^{19}F-{^1H} NMR METHODOLOGY IN SITU

The orthogonal cross-coil antenna configuration employed herein made use of a two-turn (2-cm outer diam.) space-wound surface coil tuned for ^{19}F at 188.154 MHz. The proton decoupling circuit is independent of this observation antenna; it is constructed from two 3-cm outer diam. single turn loops connected in parallel in a Helmholtz configuration tuned for ^1H at 200 MHz. Standard capacitive tuning to the resonance frequency and for a 50 Ω impedance match was employed for both circuits. Fig. 2 illustrates the relative position of the observation and decoupling coils on the head of a rat. Radio-frequency isolation is achieved by optimizing geometrical orthogonality between the observe surface coil and the Helmholtz decoupling coil with an animal in place. Typical isolation between the observe and decouple channels is 40 dB (measured at 2 W decoupling power). Additional isolation is achieved by the use of a series of high efficiency rf cavity resonator notch filters outside the bore of the magnet on both the ^{19}F observation and ^1H decouple channels (~35 dB rejection, ~0.4 dB insertion loss; Celwave, Inc., Marlboro, NJ). No increase in the noise level of the ^{19}F spectrum during ^1H decoupling was observed with this configuration.

RESULTS AND DISCUSSION

The utility of proton decoupling ^{19}F resonances in situ was investigated after intravenous administration of the glucose analogue 2FDG (200 mg/kg). The LD_{50} for this compound is ~600 mg/kg/day i.p. for five consecutive days (15). A nonfluorine containing mixture of ketamine/xylaxine (87 mg/kg:13 mg/kg) was administered intramuscularly to maintain the subject, an ~250-g male Sprague-Dawley rat, under anesthesia throughout the experiment (16).

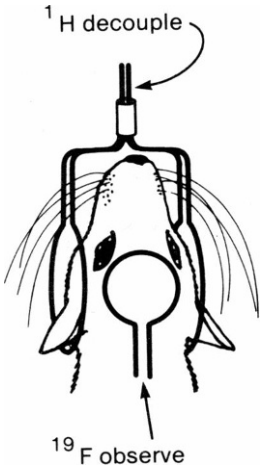

FIGURE 2 Artist's rendering, at an oblique angle, of the relative position of the ^{19}F observation surface coil and Helmholtz ^1H decoupling coil on a rat head. At this angle, the two-turn stacked surface coil appears as a single loop. Coupling between the two antennas, measured as ^1H radio frequency feedthrough on the observation channel, is minimized by optimizing geometrical orthogonality. Further reduction in coupling is achieved by physical isolation of the observation coil tuning network from the decoupling coil tuning network. This provides 40 dB of isolation between the two channels (in the absence of additional filtering, see text) with the animal in place.

All NMR experiments in vivo were performed on a 4.7-T pulsed Fourier transform NMR spectrometer (model CXP-200; Bruker Instruments, Inc., Billerica, MA) with a room temperature shim bore of 8.5-cm inner diam. Static magnetic field homogeneity was optimized in situ by shimming on the proton NMR signal of water in cerebral tissue through the observation antenna (17). Spectra were collected at 5-min intervals under broadband gated decoupling conditions in 8 K data blocks with a spectral width of 20 kHz, an observation pulse repetition period of 0.454 s, and a pulse width of 7 μs at 100 W. The proton decoupler was gated on at 3.2 W during the acquisition time (0.255 s) and then gated off for the rest of the pulse repetition period to achieve an average power of 1.8 W in order to minimize rf tissue heating effects. This decoupler gating scheme may also be expected to reduce the NOE effect. A sealed microsphere (Wilmad Glass Co., Inc., Buena, NJ) containing 0.5% hexafluorobenzene and 70 mM chromium acetylacetonate (as a relaxation agent) in benzene was used as an external reference.

Fig. 3 illustrates three consecutive ^{19}F NMR spectra of brain tissue in situ under either proton coupled (Fig. 3 *B*) or decoupled (Fig. 3, *A* and *C*) conditions taken ~2 h post injection of 2FDG. In Fig. 3 *B* only two fluorine resonances (*a* and *b*) are readily observable under proton coupled conditions where lineshapes are broadened by unresolved J_{FH}. In contrast, three additional resonances (*c*–*e*) are

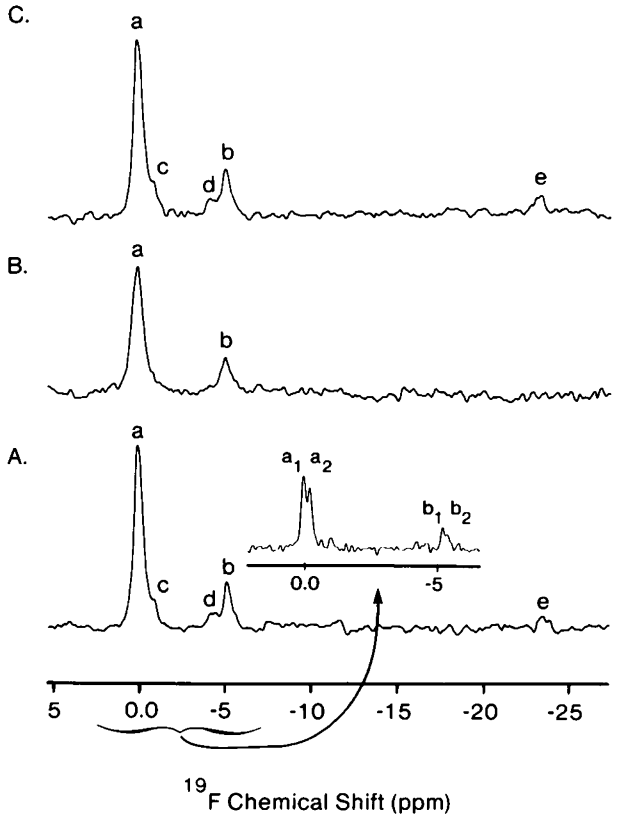

FIGURE 3 In situ cerebral ^{19}F spectra taken ~2 h after a 50-mg bolus i.v. injection of 2FDG into an ~250-g mole Sprague-Dawley rat anesthetized with a nonfluorine containing anesthetic (see text). Spectra *A–C* represent three consecutive 10-min time domain data accumulations under proton decoupled (*A* and *C*) and proton coupled (*B*) conditions. Decoupled spectra *A* and *C* were taken just before (*A*) and after (*C*) to acquisition of coupled spectrum *B*. At this point in the metabolic time course of 2FDG the proton-coupled spectrum shows only two resonances (*a* and *b*). Proton-decoupling improves spectral resolution and sensitivity revealing three additional resonances (*c–e*). Resonance *a* in *A* has been assigned to primarily 2FDG6P (see text). Nakada and Kwee (23) have identified resonance *b* as 2-fluoro-2-deoxy-glycerol, resonance *c* as 2-fluoro-2-deoxy-D-sorbitol, and resonance *d* as 2-fluoro-2-deoxy-L-glyceraldehyde; resonance *e* remains unassigned. In some subjects, an additional resonance (*f*) at +2 ppm, identified as 2-fluoro-2-deoxy-6-phosphogluconate (9, 10), is also observed. Resonance *b* in the resolution enhanced insert is labeled as two peaks (b_1 and b_2), however, due to a low signal-to-noise ratio this assessment is tentative. IUPAC chemical shift convention (22) has been followed with the chemical shift of resonance (a_1) (*A, insert*), which arises primarily from the β-anomer of 2FDG6P (see text), arbitrarily assigned 0.0 ppm in this figure.

detected under proton decoupled conditions (Fig. 3, *A* and *C*) illustrating the marked sensitivity improvement that can be obtained by collapsing the complex J_{FH} multiplet patterns (e.g., Fig. 1). For the set of spectra shown in Fig. 3, the linewidth at half height ($\Delta\upsilon_{1/2}$) for the intense resonance *a* attributable to the overlapping resonance lines of 2FDG and 2FDG6P (vide infra) decreases from ~115 Hz in the absence of ^{1}H decoupling to 80 Hz in the presence of ^{1}H decoupling. The signal-to-noise ratio for this resonance increased approximately 45% with proton decoupling. This increased sensitivity and linewidth resolution allows post facto resolution enhancement techniques to be used to good advantage for structure-shift assignments. For example, as is seen in Fig. 3 *A, insert*, Lorentzian-to-Gaussian lineshape transformation (18) resolves the intense fluorine resonance (*a*) (2FDG and 2FDG6P) into two peaks (a_1, a_2) with a chemical shift difference of 0.23 ppm. Peak a_1 represents the overlapping β-anomer resonances of both 2FDG and 2FDG6P while peak a_2 represents their overlapping α-anomer resonances. Qualitative peak analysis based on this chemical shift difference[1] indicates that the concentration ratio of 2FDG6P/2FDG is ~2.5:1 at this point in the time course of 2FDG metabolism. This supports the given assignment of these resonances primarily to the α- and β-anomers of 2FDG6P.

The extent of the ^{19}F-{^{1}H} NOE in vivo was measured by comparing signal intensities (peak heights and areas gave similar results with the most intense resonance, 2FDG6P) from spectra obtained with the decoupler gated off (no NOE) or left on (with NOE) during the "relaxation period" between sequential free induction decay acquisitions. A pulse repetition period of 7 s, about seven times the longest T_1 in vivo (2FDG6P), provided for near quantitative analysis (~5% error) of the NOE (19). The decoupler was left on during data acquisition to remove J_{FH} structure.

Time domain data were collected in 10-min time averaging blocks alternating between blocks with or without NOE over a 1–2 h period. Data collection was initiated 1.5 h postinjection of 2FDG (200 mg/kg, i.v.) into ketamine/xylazine anesthetized rats ($n = 2$). Except for the extended pulse repetition period, the acquisition parameters and decoupler power level were the same as employed previously.

For each of the two animals examined, all data blocks with NOE were summed (to improve signal-to-noise) and ^{19}F signal intensities were compared with those from the summed data blocks taken without NOE. Each of the four resonances quantified exhibited a substantial positive NOE. The ^{19}F-{^{1}H} NOE was most accurately measured for the dominant 2FDG6P resonance as 21.5 and 23%. The other less intense ^{19}F resonances showed similar enhancements yielding NOE values of 14 and 41% for peak *b*, 15 and 30% for peak *d*, and 14 and 28% for peak *f* (assignments as in Fig. 3).

In practice we find the implementation of ^{19}F-{^{1}H} to be routine once sufficient isolation between the observe and decouple circuits have been achieved. The new multipulse ultra-low power decoupling schemes (20) can be employed to further decrease tissue heating from that obtained with gated decoupling. Although geometrically orthogonal coils can be readily adopted to monitor fluorine chemistry in

[1]NMR spectral simulation was employed to map the observed net α, β anomer peak separation [i.e., $\delta_\beta(a_1) - \delta_\alpha(a_2)$] vs. relative distributions of 2FDG and 2FDG6P. From this theoretically derived "calibration curve," a measurement of peak separation in vivo yields the concentration ratio of 2FDG6P/2FDG.

other tissue in addition to brain, use of "electrically orthogonal" coil designs may in some instances prove advantageous. For example, use of a circular surface coil for ^{19}F observation in conjunction with a coaxial "figure eight" coil for ^{1}H decoupling may eliminate the restrictions of geometrical orthogonality while maintaining sufficient rf isolation between the two channels (21).

CONCLUSION

In summary, the substantial advantages of proton decoupling the fluorine nuclide in functioning cerebral tissue in situ have been demonstrated in a standard high-field, vertical wide-bore, high-resolution NMR spectrometer using an orthogonal cross-coil antenna configuration. When $J_{FH} \geq \Delta\nu_{1/2}$(tissue), this methodology provides substantial improvements in the spectral resolution and signal-to-noise of the fluorine spectrum from collapse of the scalar coupling patterns. Additional sensitivity enhancement can be obtained from a positive NOE. We are currently applying ^{19}F-$\{^{1}H\}$ NMR in the study of 2- and 3FDG metabolism in cerebral tissue in situ.

Helpful discussions with Ruthmary K. Deuel and assistance from Seong-Gi Kim are gratefully acknowledged.

This work was supported by National Institutes of Health grant GM-30331 and National Science Foundation instrument grant CHE-8100211.

Received for publication 24 June 1986 and in final form 25 November 1986

REFERENCES

1. Ackerman, J. J. H., T. H. Grove, G. G. Wong, D. G. Gadian, and G. K. Radda. 1980. Mapping of metabolites in whole animals by ^{31}P NMR using surface coils. *Nature* (*Lond.*). 283:167–170.
2. Stevens, A. N., P. G. Morris, R. A. Iles, P. W. Sheldon, and J. R. Griffiths. 1984. 5-Fluorouracil metabolism monitored in vivo by ^{19}F NMR. *Br. J. Cancer.* 50:113–117.
3. Wyrwicz, A. M., M. H. Pszenny, J. C. Schofield, P. C. Tillman, R. E. Gordon, and P. A. Martin. 1983. Noninvasive observation of fluorinated anesthetics in rabbit brain by fluorine-19 nuclear magnetic resonance. *Science* (*Wash. DC*) 222:428–430.
4. Bell, R. A., and J. K. Saunders. 1970. Heteronuclear ^{1}H-^{19}F nuclear Overhauser effects in fluoroaromatic compounds. *Chem. Comm.* (*J. Chem. Soc. Sect. D*) 1078.
5. Hull, W. E., and B. D. Sykes. 1975. Dipolar nuclear spin relaxation of ^{19}F in multispin systems. *J. Chem. Phys.* 63:867–880.
6. Revich, M., D. Kuhl, J. Greenburg, M. E. Phelps, T. Ido, V. Cassella, J. Fowler, A. Alavi, and L. Sokoloff. 1979. The ^{18}F-fluorodeoxyglucose method for the measurement of local cerebral glucose utilization in man. *Circ. Res.* 44:127–137.
7. Phelps, M. E., S. C. Huang, E. J. Hoffman, C. S. Selin, L. Sokoloff, and D. E. Kuhl. 1979. Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18) 2-fluoro-2-deoxyglucose: validation of method. *Ann. Neurol.* 6:372–388.
8. Fox. J. L. 1984. PET Scan controversy aired. *Science* (*Wash. DC*) 224:143–144.
9. Nakada, T., I. L. Kwee, and C. B. Conboy. 1986. Noninvasive in vivo demonstration of 2-fluoro-2-deoxy-D-glucose. D-glucose metabolism beyond the hexokinase reaction in rat brain by ^{19}F nuclear magnetic resonance spectroscopy. *J. Neurochem.* 46:198–201.
10. Wyrwicz, A. M., R. Murphy, I. Prakash, R. M. Moriarty, and T. Dougherty. 1985. 2-Fluoro-2-deoxy-D-glucose metabolism in rat brain. *Annu. Meeting Soc. Magn. Res. Med., 4th, Lond.* 827–828. (Abstr.)
11. Berkowitz, B. A., and J. J. H. Ackerman. 1985. 2-Fluoro-2-deoxy-D-glucose (2FDG) metabolism in vivo: a ^{19}F-$\{^{1}H\}$ NMR Study. *Annu. Meeting Soc. Magn. Res. Med., 4th, Lond.* 759–760. (Abstr.)
12. Babcock, E. E., and R. L. Nunnally. 1985. In vivo and in vitro determination of 2-fluoro-deoxyglucose by F-19 NMR imaging and spectroscopy. *Annu. Meeting Soc. Magn. Res. Med., 4th, Lond.* 751–752. (Abstr.)
13. Phillips, L., and V. Wray. 1971. Stereospecific electronegative effects. Part I. The ^{19}F nuclear magnetic resonance spectra of deoxyfluoro-D-glucopyranoses. *J. Chem. Soc. (Lond.) (B).* 1618–1624.
14. Bessell, E. M., and P. Thomas. 1973. The deoxyfluoro-D-glucopyranose 6-phosphates and their effect on yeast glucose phosphate isomerase. *Biochem. J.* 131:77–82.
15. Bessell, E. M., V. Doreen Courtenay, A. B. Foster, M. Jones, and J. H. Westwood. 1973. Some in vivo and in vitro antitumour effects of the deoxyfluoro-D-glucopyranoses. *Europ. J. Cancer.* 9:463–470.
16. Van Pelt, L. F. 1977. Ketamine and xylazine for surgical anethesia in rats. *J. Am. Vet. Med. Assoc.* 171:842–844.
17. Ackerman, J. J. H., D. G. Gadian, G. K. Radda, and G. G. Wong. 1981. Observation of ^{1}H NMR signals with receiver coils tuned for other nuclides. *J. Magn. Res.* 42:498–500.
18. Ferrige, A. G., and J. C. Lindon. 1978. Resolution enhancement in FT NMR through the use of a double exponential filter. *J. Magn. Res.* 31:337–340.
19. Opella, S. J., D. J. Nelson, and O. Jardetzky. 1976. Dynamics of nuclear Overhauser enhancement in proton decoupled carbon-13 nuclear magnetic resonance. *J. Chem. Phys.* 64:2533–2535.
20. Shaka, A. J., J. Keller, and R. Freeman. 1983. Evaluation of a new broad-band decoupling sequence: WALTZ-16. *J. Magn. Res.* 53:313–340.
21. Battocletti, J. H., R. E. Halbach, A. Sances Jr., S. J. Larson, R. L. Bowman, and V. Kudravcev. 1979. Flat crossed coil detector for blood flow measurement using nuclear magnetic resonance. *Med. Biol. Eng. Comput.* 17:183–191.
22. IUPAC Recommendations on NMR Spectra. 1976. *Pure Appl. Chem.* 45:219.
23. Nakada, T., and I. L. Kwee. 1986. In vivo metabolism of 2-fluoro-2-deoxy-D-glucose in the aldose reductase sorbitol pathway in rat brain demonstrated by ^{19}F NMR spectroscopy. *Biochem. Arch.* 2:53–61.