Determination of cerebral glucose transport and metabolic kinetics by dynamic MR spectroscopy


Van Zijl, P. C. M., D. Davis, S. M. Eleff, C. T. W. Moonen, R. J. Parker, and J. M. Strong. Determination of cerebral glucose transport and metabolic kinetics by dynamic MR spectroscopy. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1216–E1227, 1997.—A new in vivo nuclear magnetic resonance (NMR) spectroscopy method is introduced that dynamically measures cerebral utilization of magnetically labeled [1-13C]glucose from the change in total brain glucose signals on infusion. Kinetic equations are derived using a four-compartment model incorporating glucose transport and phosphorylation. Brain extract data show that the glucose 6-phosphate concentration is negligible relative to glucose, simplifying the kinetics to three compartments and allowing direct determination of the glucose-utilization half-life time \( t_1 = \ln 2/(k_2 + k_3) \) from the time dependence of the NMR signal. Results on isofluorane \( (n = 5) \) and halothane \( (n = 7) \)-anesthetized cats give a hyperglycemic \( t_1 = 5.10 \pm 0.11 \text{ min}^{-1} \) (SE). Using Michaelis-Menten kinetics and an assumed half-saturation constant \( K_i = 5 \pm 1 \text{ mM} \), we determined a maximal transport rate \( T_{\text{max}} = 0.83 \pm 0.19 \text{ mmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \), a cerebral metabolic rate of glucose \( \text{CMR}_{\text{Glc}} = 0.22 \pm 0.03 \text{ mmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \), and a normoglycemic cerebral influx rate \( \text{CIR}_{\text{Glc}} = 0.37 \pm 0.05 \text{ mmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \). Possible extension of this approach to positron emission tomography and proton NMR is discussed.

[13C]glucose utilization; brain; Michaelis-Menten kinetics; cat; nuclear magnetic resonance spectroscopy

GLUCOSE IS THE PRIMARY fuel for energy metabolism in normal brain, and the availability of noninvasive in vivo methods for the elucidation of its transport and metabolic kinetics should be important in the study of a multitude of brain disorders (17, 21). An ideal method for measuring cerebral rates of influx (CIR\(_{\text{Glc}}\)) and metabolism (CMR\(_{\text{Glc}}\)) should allow direct monitoring of the separate processes of tissue uptake and utilization of glucose under physiological conditions. At present, the most sensitive in vivo approach is the use of radiolabels, which can be applied in tracer amounts (9, 10, 14, 23, 26, 27). However, the interpretation of these measurements is complicated by the fact that the sum of all radiolabels, which basically includes all metabolic products, is measured (13). This problem has been addressed by using glucose derivatives that are trapped after phosphorylation, e.g., deoxyglucose analogs (14, 26, 27), but these compounds may differ in their transport and utilization properties with respect to glucose. This difference is generally accounted for empirically by the use of so-called lumped constants in positron emission tomography (PET) (7, 22). It has been suggested that the kinetic equations for deoxyglucose may need to account for the potentially reversible character of saturable phosphorylation (14), but irreversibility is generally used as a reasonable assumption.

An alternative approach that can be applied to study glucose utilization without the need for a lumped constant is the nuclear magnetic resonance (NMR) study of nonradioactive magnetically labeled glucose, e.g., the naturally occurring isotopomer [1-13C]glucose (3, 4, 11, 30). In principle, NMR can study both the uptake (influx) and metabolism of [1-13C]glucose (11, 18, 30), because different metabolites labeled in different carbon positions generally result in different NMR spectral frequencies (chemical shifts). This specific labeling has been studied by 13C NMR in animals and humans (2, 4, 11). Indirect determination of transport kinetics was recently also achieved by applying equilibrium Michaelis-Menten kinetics to the experimentally measured ratios of plasma and tissue glucose (11). However, NMR is an insensitive method with several inherent problems that interfere with optimum use of its enormous potential. For instance, the time necessary to acquire a 13C NMR spectrum is much longer than the typical time constant for glucose uptake and phosphorylation, thereby prohibiting direct kinetic determination of the rates of glucose influx and utilization. The sensitivity of 13C NMR can be improved by...
detecting the proton nuclei coupled to the labeled carbon nuclei (8, 24), but until recently this approach prohibited detection of $[1^{-13}C]$glucose due to its overlap with the dominant water resonance (108 M proton concentration due to 2 protons/water molecule vs. millimolar concentration for the glucose). Therefore, this $[1^{-13}C]$glucose transport step has not yet been studied dynamically by $^{13}C$ NMR, and assumptions for this part of the metabolic process have to be made when formation of $[^{13}C]$glutamate (18) and $[^{13}C]$lactate (25) is studied. We have recently developed proton NMR methods that can simultaneously detect all metabolites, even when they are resonating at the same frequency as water (29), and we apply this method here to dynamically study uptake and metabolism of $[1^{-13}C]$glucose in healthy cat brain. This approach is the closest possible NMR analog of a PET experiment, since we can limit the detected signal to consist of glucose and phosphorylated glucose only. NMR is not able to use fluorodeoxyglucose due to its toxicity when used in the amounts required for NMR (5).

**Glossary**

- **BBB** Blood-brain barrier
- $C_p$, $C_{p}^*, C_{p}^{ext}$ Unlabeled ($^{12}C$), labeled ($^{13}C$), and total plasma glucose concentrations (mM)
- $C_E$, $C_{E}^*$, $C_{E}^{ext}$ Unlabeled, labeled, and total tissue glucose concentrations (mM)
- $C_M$, $C_{M}^*$, $C_{M}^{ext}$ Unlabeled, labeled, and total phosphorylated glucose concentrations in brain tissue (mM)
- $C_i$ Sum of phosphorylated and tissue glucose concentrations (mM)
- $C_{Glc}$ Total concentration of glucoses (mM): $C_{Glc} = C_p + C_E + C_M$
- $C_{blood}$ Arterial blood glucose concentration (mg/dl)
- $C_{X,eq}$ Concentrations at equilibrium
- $CMRGlc$ Cerebral metabolic rate of glucose ($\mu$mol·g$^{-1}$·min$^{-1}$)
- $CIR_{Glc}$ Cerebral influx rate of glucose ($\mu$mol·g$^{-1}$·min$^{-1}$)
- $f_{gas}$ Vascular volume fraction
- GC-MS Gas chromatography-mass spectroscopy
- Hct Hematocrit
- HMQC Heteronuclear multiple quantum coherence
- $K_1$ Michaelis-Menten half-saturation constant (mM) for glucose transport
- $K_{hase}$ Michaelis-Menten half-saturation constant for glucose phosphorylation (hexokinase-catalyzed = hase)
- $k_1$ Rate constant for glucose influx in the brain (min$^{-1}$)
- $k_2$ Rate constant for glucose efflux from the brain (min$^{-1}$)
- $k_3$ Rate constant for glucose phosphorylation in brain tissue (min$^{-1}$)
- $k_4$ Rate constant for hydrolysis (dephosphorylation) of glucose phosphate (min$^{-1}$)
- $k_5$ Rate constant for metabolism of glucose phosphate (min$^{-1}$)
- MRS Magnetic resonance spectroscopy
- MRI Magnetic resonance imaging
- NMR Nuclear magnetic resonance

**PET** Positron emission tomography
**$T_{max}$** Michaelis-Menten maximum transport rate of glucose ($\mu$mol·g$^{-1}$·min$^{-1}$)
**$T_0$** Time necessary for labeled glucose to reach brain after start of infusion (min)
**$t_0$** Half-life time for glucose utilization [$ln2/ (k_2 + k_3)$]
**$V_d$** Whole brain water volume (ml/g)

**MATERIALS AND METHODS**

Metabolic model. The model presented here is based on Sokoloff’s $[^{14}C]$deoxyglucose model (14, 27) developed for autoradiography. It is extended to include the specific assumptions necessary for our NMR experiments. For instance, although tracer kinetics do not apply, it is assumed that first-order kinetics are still valid because the observed processes are unsaturable. Second, $[1^{-13}C]$glucose is a naturally occurring isotopomer of glucose, and it is generally assumed that the kinetic constants are the same as for the nonmagnetic isotopomer $[^{12}C]$glucose, thereby avoiding the need for a lumped constant. We would like to point out that this assumption is not trivial, because atoms of different weight and different size react equivalently chemically, but may do so at a different rate. This effect is actually used by archaeologists to study evolution from the behavior of carbon isotopes during photosynthesis (for a review of this literature see Ref. 28). However, although this effect is significant (a few percent) for CO$_2$ fixation, it is expected to be negligible for the glucose transport and metabolism steps studied here. The reasons are that the bond to the isotope is not broken and that the change from $^{12}C$ to $^{13}C$ should not influence the size ($M = 181$) and shape of the glucose unit significantly for an enzyme to distinguish between the two isotopes. Thus the general assumption in NMR and PET that isotopic labeling of a single carbon atom does not influence the enzyme kinetics of transport and metabolism is very plausible.

To describe the experiment completely, the model needs four compartments (Fig. 1), namely a blood compartment, a compartment for glucose in tissue, a compartment for physiology.
phosphorylated glucose in tissue, and a compartment for metabolic products synthesized after phosphorylation. The boundary between the first two compartments consists of the capillary and cell membranes (BBB). Following Sokoloff et al. (26, 27), the capillary glucose concentration will be approximated by the arterial plasma glucose concentration. With the assumption of rapid equilibration of glucose over the cell membranes (1, 17) or, equivalently for the model, visualizing the capillary and cell membrane as a single barrier, the next boundary is the hexokinase-catalyzed phosphorylation of glucose. Because the [13C]glucose utilization is not saturable, it is not necessary to include the effect of hydrolysis of phosphorylated glucose back to glucose, and it is accurate to assume a zero rate constant $k_5$ for this step. To account for efflux of phosphorylated glucose into different products, a fourth compartment is introduced, described by the single rate constant $k_5$. This approach is supported by data from direct 13C NMR spectroscopy at equilibrium (2, 4), which show that the concentrations of any intermediates between phosphorylated glucose in tissue, and a compartment for metabolic products synthetized after phosphorylation. The boundary between the first two compartments consists of the capillary and cell membranes (1, 17) or, equivalently for the model, visualizing the capillary and cell membrane as a single barrier, the next boundary is the hexokinase-catalyzed phosphorylation of glucose. Because the [13C]glucose utilization is not saturable, it is not necessary to include the effect of hydrolysis of phosphorylated glucose back to glucose, and it is accurate to assume a zero rate constant $k_5$ for this step. To account for efflux of phosphorylated glucose into different products, a fourth compartment is introduced, described by the single rate constant $k_5$. This approach is supported by data from direct 13C NMR spectroscopy at equilibrium (2, 4), which show that the concentrations of any intermediates between total [1-13C]glucose and [4-13C]glutamate are negligible, indicating rapid conversion to each next step. Thus consecutive metabolism after phosphorylated glucose in healthy brain can be described by a single rate constant $k_5$ to [4-13C]glutamate. In this respect, it is important to notice that it is indifferent for our model if the step to the first product after phosphorylation is indifferent for our model if the step to the first product after phosphorylation. In this respect, it is important to notice that it is indifferent for our model if the step to the first product after phosphorylation. The complete expression describing the combined changes of glucose in plasma and tissue plus G-6-P in tissue can be obtained from combining Eqs. 4, 6, 7, and 8 in the following expressions at equilibrium

\[
\frac{dC_{\text{Glc}}}{dt} = \frac{k_1 k_3}{(k_2 + k_3)} C_{\text{Glc}} \left[ (1 - e^{-k_2 T}) - k_5 \right]
\]

(1)

\[
\frac{dC_{\text{ep}}}{dt} = k_2 C_{\text{ep}} - (k_2 + k_3) C_{\text{Glc}}
\]

(2)

The solution to Eq. 1 for the tissue concentration of labeled glucose at time $T$ after introduction of [1-13C]glucose into the blood is

\[
C_{\text{Glc}}(T) = k_3 e^{-(k_2+k_3)T} \int_0^T C_{\text{Glc}}(t) e^{(k_2+k_3)t} dt
\]

(3)

In our [1-13C]glucose infusion protocol, we rapidly (within 0.5-2 min) raise the total plasma glucose concentration ($C_{\text{Glc}}(0) = C_{\text{p}} + C_{\text{Glc}}$) to the desired hyperglycemic level (20–30 mM) and keep it constant (at equilibrium) for the remainder of the experiment. Thus the concentration of labeled glucose can be taken constant at any point in time, and $C_{\text{Glc}}(t) = C_{\text{Glc,eq}}$. The integral in Eq. 3 can then be evaluated for any time $T$

\[
C_{\text{Glc}}(T) = \frac{k_1 k_3}{(k_2 + k_3)} C_{\text{Glc,eq}} \left[ (1 - e^{-k_2 T}) - k_5 \right]
\]

(4)

Analogously to Eqs. 1 and 3, the solution for the concentration of phosphorylated glucose at any time point $T$ is given by

\[
C_{\text{ep}}(T) = k_3 e^{-k_3 T} \int_0^T C_{\text{p}}(t) e^{k_3 t} dt
\]

(5)

Substitution of Eq. 4 into Eq. 5, evaluation of the integral and rearrangement of the expression in terms of a common denominator leads to the following result

\[
C_{\text{p}}(t) = \frac{k_1 k_3}{(k_2 + k_3)(k_2 + k_3 - k_3 k_5)} C_{\text{Glc,eq}} \left[ (1 - e^{-k_2 T}) - k_5 (1 - e^{-k_3 T}) \right]
\]

(6)

Although most compounds detected by NMR have different chemical shifts in the proton or carbon frequency range, spectral resolution does not allow in vivo separation of the signals of blood and tissue glucose and glucose 6-phosphate (G-6-P). Thus the operational equations have to describe the concentration $C_{\text{Glc}}$ which is the sum of the contributions of labeled tissue glucose and G-6-P ($C_{\text{Glc}}$) and labeled plasma glucose, corrected for the vascular-tissue volume fraction $f_{\text{vas}}$

\[
C_{\text{Glc}}(t) = (1 - f_{\text{vas}}) C_{\text{Glc}}(t) + f_{\text{vas}} C_{\text{Glc}}(t)
\]

(7)

Finally, to describe the change of the labeled tissue plus plasma glucose, $C_{\text{Glc}}$, as a function of time from the start of infusion to equilibrium, the expressions at equilibrium ($dC_{\text{Glc}}/dt = dC_{\text{Glc,eq}}/dt = 0$) have to be evaluated. Combination of Eqs. 1, 2, and 7 leads to the following two expressions at equilibrium

\[
C_{\text{Glc,eq}} = \left[ \frac{k_1 (k_3 + k_5) + f_{\text{vas}} [k_2(k_2 + k_5) - k_3(k_2 + k_3)]}{k_3(k_2 + k_5)} C_{\text{p,eq}} \right]
\]

(8a)

\[
C_{\text{ep,eq}} = \frac{k_3(k_3 + k_5)}{k_5(k_2 + k_3)} C_{\text{p,eq}}
\]

(8b)

The complete expression describing the combined changes of glucose in plasma and tissue plus G-6-P in tissue can be obtained from combining Eqs. 4, 6, 7, and 8

\[
C_{\text{Glc}}(T) = \frac{C_{\text{Glc,eq}}}{k_3(k_2 + k_3)} \left[ (1 - f_{\text{vas}}) k_1 k_3 [k_2(k_2 + k_3) + f_{\text{vas}} k_2(k_2 + k_3)] \right]
\]

(9a)

\[
\cdot \left[ (1 - f_{\text{vas}}) k_2 [k_2 - k_3] (1 - e^{-k_2 T - T_0}) \right]
\]

(9b)

In this equation a correction time delay $T_0$ has been added to adjust for the delay time between the start of the infusion and the time that glucose reaches the brain, which basically is the intercept of the experimental curve with the time axis. Equation 9a shows that the experimental curve can be fitted...
independently of knowledge of the actual plasma and tissue glucose concentrations by calibrating the signal intensities with respect to the signal intensity at equilibrium. The curve has to be fitted to four rate constants. The time intercept \( T_0 \) is well known from the experimental arterial plasma data and is not an additional variable. If the plasma contribution to the spectrum can be neglected \( (f_{vas} = 0) \), the curve represents the combined tissue glucose and has to be fitted to only three rate constants

\[
C^*(T) = \frac{C^{eq*}_{vas}}{(k_3 + k_0)(k_2 + k_3 - k_0)} \left[ k_2(k_2 - k_3)[1 - e^{-(k_2 + k_3)(T - T_0)}] + k_3(k_2 + k_3)[1 - e^{-k_3(T - T_0)}] \right] \tag{9b}
\]

Equations 9a and 9b are the exact operational equations to be used for fitting the experimental data to a four-compartment model. A quick check of their accuracy is provided by calculating the limit for equilibrium between inflow and metabolism: for long times \( T \), all rate constants and vascular fractions drop out.

Determination of rate constants from the proton-detected \(^{13}\)C NMR spectra. To obtain the rate constants and \( T_0 \), the experimental glucose uptake and phosphorylation curve has to be fitted to Eq. 9a or 9b. Fitting of a single experimental curve to three or four rate constants may lead to a large variance in the results. This variance can be reduced if additional restrictions and/or reasonable starting values are available for the variables. To simplify the problem, it would be very convenient to know the concentration of phosphorylated glucose to pyruvate do not have protons connected to \(^{13}\)C and are therefore not detected. Second, \([3-^{13}\)C]pyruvate has the proton resonance at 2.4 ppm and its \(^{13}\)C resonance at 28.5 ppm. The first experimental (Fig. 2) proton signal appearing in addition to the total glucose resonances in healthy brain is at 2.4 ppm and could in

\[
C_{glc}(T) = (1 - f_{vas})C_T(0) + f_{vas}C^*_T(0) \tag{10}
\]

\[
C^*_E, eq = \frac{k_1}{(k_2 + k_3)} C^*_E, eq \tag{11}
\]

\[
C^*_E(0) = C^*_E, eq[1 - e^{-(k_2 + k_3)(T - T_0)}] \tag{12}
\]

The above equations describe the rate equations for labeled glucose only, which apply to our present method for gradient-enhanced proton-detected \(^{13}\)C spectroscopy in which we only detect compounds that have one or more protons directly connected to a labeled carbon nucleus. However, it should still be shown that we detect \([1-^{13}\)C]glucose only and not any other metabolites. As mentioned, most compounds detected by NMR spectroscopy have different chemical shifts in the proton or carbon frequency range and can be detected separately (30). Although spectral resolution does not allow in vivo separation of the signals of blood and tissue \([1-^{13}\)C]glucose and \([1-^{13}\)C]G-6-P, the total glucose signals (4.6 and 5.4 ppm) can be separated from all other brain metabolic glucose products that are present in sufficient concentration during our 30- to 40-min experiment (Fig. 2). First of all, the metabolic products in the subsequent series of reactions from phosphorylated glucose to pyruvate do not have protons connected to \(^{12}\)C and are therefore not detected. Second, \([3-^{13}\)C]pyruvate has the proton resonance at 2.4 ppm and its \(^{13}\)C resonance at 28.5 ppm. The first experimental (Fig. 2) proton signal appearing in addition to the total glucose resonances in healthy brain is at 2.4 ppm and could in

\[
\alpha-[1-^{13}\)C]-glucose
\]

\[
\beta-[1-^{13}\)C]-glucose
\]

\[
[4-^{13}\)C]-glutamate
\]

\[
\alpha-[1-^{13}\)C]-glucose
\]

\[
[4-^{13}\)C]-glutamate
\]

\[
\alpha-[1-^{13}\)C]-glucose
\]

\[
[4-^{13}\)C]-glutamate
\]

\[
\alpha-[1-^{13}\)C]-glucose
\]

\[
[4-^{13}\)C]-glutamate
\]

Fig. 2. A: proton-detected \(^{13}\)C spectra (each spectrum 1 min 24 s) showing spectral changes during infusion. All spectra are difference spectra with respect to preinfusion. Infusion was started after the 4th displayed spectrum. Resonances at 5.4 and 4.6 ppm are for \( \alpha \)- and \( \beta \)-isomers of \([1-^{13}\)C]glucose, respectively. Each signal is a doublet (170 Hz splitting). The very small signals just appearing at 2.4 ppm are from 1st metabolic product, \([4-^{13}\)C]glutamate. For experiment we use total glucose signal. B: comparison of normalized (with respect to equilibrium: 100\%), total arterial glucose levels (\( \triangle \)) and spectral glucose intensities (\( \bullet \)). C: brain glucose uptake curve in concentration units, determined using the procedure outlined in MATERIALS AND METHODS. Result of data fitting to Eq. 12 and dashed line used to determine \( k_1 \) from initial rate are also displayed.
principle be \([3^{-13}C]\)pyruvate (15) or \([4^{-13}C]\)glutamate-glutamine (24, 29). As mentioned above, combined proton and carbon studies have shown the first detectable product to be \([4^{-13}C]\)glutamate (2, 4, 8, 29). Thus our experiment measures total labeled glucose \(C_{\text{Glc}}(T)\).

Two cases can now be distinguished, one in which data interpretation is straightforward and one requiring an additional assumption. If the vascular contribution is negligible, spectral intensities can be scaled with respect to the equilibrium spectral intensities and the resulting exponential can be fitted to Eq. 12, allowing direct determination of the tissue glucose half-life time without additional assumptions

\[
\tau_{\text{eq}} = \frac{\ln 2}{k_2 + k_3} \quad (13)
\]

If the vascular contribution is not negligible, the experimental NMR signal intensities represent \(C_{\text{Glc,eq}}\) from the equilibrium ratio of plasma and tissue glucose reported in the literature and the experimentally determined plasma level for labeled glucose \(C_{\text{P,eq}}\). After the \(C_{\text{Glc}}(T)\) curve is normalized with respect to \(C_{\text{Glc,eq}}\), the correct \(C_{\text{Glc}}(T)\) values can be obtained using Eq. 10 and the experimental \(C_{\text{Glc}}(T)\) values. The final \(C_{\text{Glc}}(T)\) curve can then be fitted to Eq. 12.

In RESULTS and DISCUSSION, we will evaluate the influence of the number of unknowns in Eq. 10 to three but does not reduce the number of unknowns in Eq. 9a. As a matter of fact, Eqs. 9a and 9b become equal if Eq. 7 is used to correct the experimental data for the assumed tissue-to-plasma ratio and the vascular volume.

Finally, once the tissue glucose turnover rate \((k_2 + k_3)\) is determined, the rate constant \(k_1\) can be calculated from Eq. 11. Another approach of determining \(k_1\) is by initial rate determination using the initial slope (time derivative of the early points) of the experimentally determined \(C_{\text{Glc}}(T)\) curve. Assuming no phosphorylation or efflux in the early minutes, the working equation for initial rate experiments can be derived from

\[
\frac{dC_{\text{P,eq}}(t)}{dt} = k_1C_{\text{P,eq}} \quad (14)
\]

The solution for this equation is

\[
C_{\text{Glc}}(T) = k_1C_{\text{P,eq}}^T \quad (15)
\]

For our case of negligible phosphorylation, we determine \(k_1\) both from Eq. 11 and from the initial rate approximation and compare the results and accuracies for different vascular fractions and plasma-to-tissue ratios. In the general case including phosphorylation, determination of \(k_1\) by the initial rate approach reduces the situation to two equations (Eqs. 8b and 9b) and three unknowns, which may in some cases be fitted accurately.

At equilibrium, the rate constants are related to the glucose influx \((\text{CMR}_{\text{Glc}})\) and utilization \((\text{CMR}_{\text{Glu}})\) rates in micromoles per gram per minute by

\[
\text{CMR}_{\text{Glc}}/V_d = k_1C_{\text{P,eq}}^{\text{tot}} - k_2C_{\text{E,eq}}^{\text{tot}} \quad (16)
\]

\[
\text{CMR}_{\text{Glu}}/V_d = k_3C_{\text{E,eq}}^{\text{tot}} - k_2C_{\text{E,eq}}^{\text{tot}} \quad (17)
\]

These parameters, describing the facilitated inflow of glucose and its metabolism, respectively, are equivalent to the quantities \(V_{\text{in}}\) and \(V_{\text{ex}}\) used by Mason et al. (18) in their evaluation of glutamate production by the trichloroacetic acid cycle. Equation 17 also indicates that, in the present model at equilibrium, the effective glucose transport rate \((k_1C_{\text{P,eq}}^{\text{tot}} - k_2C_{\text{E,eq}}^{\text{tot}})\) is equal to the phosphorylation rate.

Data processing protocol. In our experiments, the NMR signal intensity of total \([1^{-13}C]\)glucose and the total arterial blood glucose concentration (glucose analyzer) are measured as a function of time. The data are processed as follows.

1) The plasma glucose concentrations before infusion \((C_{\text{Glc}}^{\text{tot}} = C_{\text{Glc}})\) and at the plateau phase (6–10 points) of the total glucose curve \((C_{\text{Glc}}^{\text{tot}} = C_{\text{Glc}}^{\text{tot}} + C_{\text{P,eq}})\) are calculated from the experimentally determined total arterial blood glucose concentrations \((C_{\text{Glc}}^{\text{tot}})\) and hematocrits (Hct)

\[
C_{\text{Glc}}^{\text{tot}} (\text{mM}) = C_{\text{Glc}}^{\text{tot}} (\text{mg/dl}/18.1[1 - (\text{Hct/100})]) \quad (18)
\]

in which 18.1 is the molar mass of \([1^{-13}C]\)glucose divided by 10 to correct for the l/dl conversion. These concentrations are then used to determine the enriched plasma glucose concentration \(C_{\text{P,eq}}\) at each measured time point.

2) The enriched plasma glucose level \(C_{\text{P,eq}}\) and the rat plasma-to-tissue ratio of glucose \((C_{\text{P,eq}}/C_{\text{E,eq}} = 2.6)\) are used to calculate the enriched tissue glucose concentration at equilibrium. The total tissue plus plasma glucose concentration in millimolar units for the plateau phase of the curve is determined using Eq. 10 and a specified vascular fraction.

3) The complete experimental NMR signal intensity curve is then calibrated in terms of a \(C_{\text{Glc}}(T)\) in millimolar and in terms of a \(C_{\text{Glc}}(T)\) in millimolar units, as described in Determination of rate constants from the proton-detected \([1^{-13}C]\)NMR spectra.

4) The time constant \(T_{\text{eq}}\) is taken as the time that it takes for the small residual gradients by preemphasis. The so-called gradi-
ent-enhanced HMQC pulse sequence that was used has been described in detail elsewhere, and the parameters that were applied were exactly as described previously (29). In short, gradient lengths were 2 ms; postgradient delays of 300 µs were used for recovery. Additional gradients (G_ADD) of 0.06 and 0.04 T/m were used in the x and z directions. In some cats we used combined coherence selection gradient ratios G1:G2:G3 = 3:5:0 and 2:2:1 in different gradient directions, which select for the same heteronuclear coherence pathway. In other cases we only used 3:5:0 in two or three gradient directions. Variation of these selection gradients does not influence our spectra (29). A two-step phase cycle was used in addition to gradient selection, resulting in proton spectra that are free of water (Fig. 2). It is important to notice that, since only one of two coherence pathways is selected, spectra generally cannot be phased and have to be processed as magnitude spectra.

The experimental protocol was as follows. After initial spin-echo imaging to determine the slice position and before the start of infusion, hardware and sequence performance were always tested on a 100 mM [1-13C]glucose phantom placed on top of the coil. For this test, slice selection was moved from the cat (below the coil) to the phantom. After the test, slice position and width and proton pulse widths were optimized on the cat brain and the slice was shimmed using the water spin-echo. The initial proton pulse width was calculated from the in vivo proton pulse width using the phantom proton-to-carbon pulse-width ratio. This initial value always proved to be close to correct. Radio frequency pulse widths generally did not vary more than 5–10% per cat experiment. Data were processed with a line broadening of 13 Hz. In different experiments, the number of scans acquired per spectrum varied from 64 to 128, with different total scan times ranging between 1 and 2.5 min. Before the start of the infusion, 10–12 reference spectra were acquired, which were later added and normalized to the intensity of the single spectra. This reference spectrum was subtracted from the data acquired during infusion (difference spectroscopy). The purpose of the high number of reference spectra is to reduce data acquired during infusion (difference spectroscopy). The purpose of the high number of reference spectra is to reduce

Animal preparation. Animals were handled in accordance with the standards established by the US Animal Welfare Acts, set forth by the National Institutes of Health (NIH) guidelines and guidelines of the John Hopkins University Animal Care and Use Committee. There were two groups of cats, namely those studied at NIH (cats 1-5) and those studied at John Hopkins University (cats 6-12), for which the anesthetic protocols were slightly different. Fasted cats (adults, about 3.0 kg, either sex) were initially anesthetized with ketamine (cats 1-5, 44 mg/kg ip) or pentobarbital sodium (cats 6-12, 40 mg/kg ip). After tracheal intubation, the femoral arteries and veins were catheterized. Subsequently, the animals were immobilized with 1 mg/kg succinylcholine chloride (cats 1-5, iv) or pancuronium bromide (cats 6-12, iv) and placed under mechanical ventilation with 1% isoflurane in 30:70% O2-N2O (cats 1-5) or 0.5–1.5% halothane in oxygen-enriched air (cats 6-12). The tidal volume was adjusted to maintain PaCO2 between 30 and 40 mmHg and PaO2 > 100 mmHg. Immobilization was maintained with pancuronium bromide (0.2 mg·kg⁻¹·h⁻¹). The time between induction and the start of the infusion experiments was always at least 3 h, resulting in an anesthetic regimen of mainly isoflurane and halothane for the two groups, respectively. Arterial pH was maintained between 7.35 and 7.45, using sodium bicarbonate and sodium hydroxide. Heart rate and blood pressure were monitored throughout the experiments. After the start of the infusion, the femoral artery catheter was repositioned, and arterial blood was sampled and measured with Radiometer ABL electrodes and analyzer (Copenhagen, Denmark). Body temperature was controlled by a thermostat-controlled water-circulated heating pad. KCl for euthanasia was administered through the femoral vein catheter. The NMR coil was placed on the head, without tissue retraction.

For the glucose uptake experiments, the blood glucose level was rapidly increased from normal (60–70 mg/dl, 3.3–3.9 mM) to hyperglycemic (>250 mg/dl, 13.8 mM) within 0.5–2 min and kept constant within ~10% for the remainder of the experiment (Fig. 2B). This was achieved using an initial bolus (1.25 ml/kg) and a subsequent infusion of exponentially decreasing rate (from 1.25 ml·kg⁻¹·min⁻¹ in the first min to 0.11 ml·kg⁻¹·min⁻¹ at 25 min, after which the rate was not changed) of a solution of 0.75 M [1-13C]glucose in saline. [1-13C]glucose (99%) was obtained from Cambridge Isotopes. Blood glucose and lactate levels (YSI model 2300 Stat Glucose/Lactate Analyzer; Yellow Springs Instruments, Yellow Springs, OH) were monitored by extraction of 0.2 ml arterial blood every 0.5 min for the first 6 min of the experiment, every 2 min for the next 6 min, and every 5–10 min at later stages. Both remained stable within 10%. All cats studied responded similarly to this protocol.

Extract studies. In vivo sampling of brain tissue was performed under anesthesia. Halothane and mechanical ventilation were continued during removal of cats (n = 3) from the magnet after completion of NMR experiments. The skin and muscle were incised and reflected using electrocautery and coagulation. The bone was removed as above, and the cortex was cleaned similarly to this protocol. The cortex was cleaned using saline. The bone was removed as above, and the cortex was cleaned.
at 4°C. The supernatant was neutralized with 3 M K₂CO₃/M KOH, mixed with chelating resin (Sigma Chemical, St. Louis, MO) to remove paramagnetic metal ion contamination, and centrifuged once more. The supernatant was freeze-dried, the lyophilized brain extract was dissolved in D₂O and filtered through a 0.2 µm, and the pH was adjusted to 7. High resolution proton-decoupled [1-¹³C] NMR spectra of the extracts were recorded with a Bruker MSL-500 NMR spectrometer [repetition time: 2 or 7 s, 12 µsec (60°) pulse; sweep width 25 kHz; 32 K data blocks zero-filled to 64 K, 2 Hz exponential line broadening].

**RESULTS**

Kinetic parameters. Figure 2A shows an example of proton-detected [1-¹³C] glucose difference spectra acquired before and during infusion. Figure 2B compares the normalized arterial glucose level input function (100% at equilibrium) in this cat to the normalized changes in total proton-detected [1-¹³C] glucose NMR signal. It is clear from the difference between the two curves that the NMR signal instead represents brain tissue glucose input and not the arterial glucose input. These normalized curves were first used to determine the hyperglycemic rates \( k_2 + k_3 \) (Eq. 12) and \( k_3 \) (Eq. 11) for 12 cats, assuming negligible vascular glucose fraction (Table 1). Subsequently, the \( k_2 + k_3 \) values at vascular fractions of 3 and 5% were determined after the total-labeled glucose curves were corrected for these plasma fractions, using the procedure outlined in METHODS AND MATERIALS. The results in Table 1 show that, when normalized curves are fit, \( k_2 + k_3 \) is the same for all vascular fractions between 0 and 5%. The rate constant \( k_3 \) was determined in two different ways, namely from \( k_2 + k_3 \) using Eq. 11 and by using an initial rate approach (Fig. 2C) in which the tangent of the initial part of the fitted curve is determined experimentally. The data clearly indicate that much lower (incorrect) results are obtained for \( k_3 \) values determined by the initial rate approach when the curve is not corrected for the vascular fraction. This makes sense, since the largest errors in the curve intensity are made for small total glucose values, which are in the region where the initial rate fitting occurs. The \( k_1 \) values at 3 and 5% are equal within error, and the result of the initial-rate approach at these two fractions (Table 1: \( k_1 = 0.055 \pm 0.003 \)) agrees well within the SE with \( k_1 \) determined using the plasma-to-tissue glucose equilibrium ratio and Eq. 11 (Table 1: \( 0.052 \pm 0.001 \)). It should be noticed that this agreement is not due to the fact that the plasma-to-tissue glucose equilibrium ratio is used to correct the curves, because very different \( k_1 \) values are found for the individual cats when the \( k_1 \) numbers determined from \( k_2 + k_3 \) and from the initial rates at \( f_{\text{vas}} = 3 - 5\% \) (e.g., cats 2 and 3) are compared. To see whether the chosen plasma-to-tissue glucose ratio

<table>
<thead>
<tr>
<th>Cat No.</th>
<th>( C_{\text{tot}} ), mM</th>
<th>( C_{\text{tot}} ), mM</th>
<th>( C_{\text{tot}} ), mM</th>
<th>( k_3 + k_3 ), min⁻¹</th>
<th>( k_1 ), min⁻¹</th>
<th>( k_1 ), min⁻¹</th>
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<td>22.6</td>
<td>0.130</td>
<td>0.130</td>
<td>0.0500</td>
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<tr>
<td>3</td>
<td>20.1</td>
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<td>24.0</td>
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<td>28.9</td>
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<td>Avg.</td>
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<td>0.003</td>
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†Calculated from the mean \( k_1 \) and \( k_3 \) at 3 different vascular fractions using Eq. 11 and \( C_{P, eq}^{tot}/C_{E, eq}^{tot} = 2.6 \). Avg., average.
influences the agreement between the initial rate and total-curve determination of $k_1$. We fitted our results at two other $C_{*P,eq}/C_{*E,eq}$ ratios for a vascular fraction of 5%. The results (Table 2) show that the average $k_1$ from both approaches is again comparable.

Determination of unlabeled glucose levels in blood during the infusion protocol. To determine the plasma levels of nonenriched glucose during the infusion protocol, we used blood samples of two cats to perform mass spectroscopy. The results for both cats were similar, and Fig. 3 shows the data for one of these cats, in which blood glucose levels were ramped up quickly to $\sim 9$ mM and then more slowly increased to 16 mM (290 mg/dl). It is clear from this figure that the unlabeled ($^{12}$C) glucose level remains stable within the error of the measurement, despite the hyperglycemic level of the total blood glucose. The correspondence between the glucose analyzer and the GC-MS data is good. It is interesting that the analyzer generally gives a 10% higher value than GC-MS, but this will not significantly influence the calculations performed in calibration of the normalized experimental NMR curve.

Extract studies. Figure 4, A and B, shows extract spectra taken in vivo and postmortem. The reliability of the in vivo study is confirmed by the low level of the $[1-^{13}$C]lactate resonance at 21 ppm, which should not be confused with the acetate standard. Lactate is very high in the postmortem spectrum. Figure 4, C and D, shows close-ups for the [1-^{13}$C]glucose region, which contains resonances for $\alpha$- and $\beta$-isomers of [1-^{13}$C]glucose (at 92.9 and 96.8 ppm, respectively) and [1-^{13}$C]G-6-P (at 93.1 and 96.9 ppm, respectively). It can be seen that the contribution of [1-^{13}$C]G-6-P to total labeled glucose is negligible in both spectra, confirming the validity of the simplified approach using three compartments. To confirm the correctness of this result, we also prepared a phantom with approximately equal amounts of [1-^{13}$C]glucose and [1-^{13}$C]G-6-P (Fig. 4E). This spectrum shows that the two resonances can indeed be separated. Finally, Fig. 4F shows the relevant glucose region for a heart extract. In this organ the [1-^{13}$C]G-6-P concentration is higher than in the brain and can be clearly distinguished. However, on the basis of the relative integrals of the two resonances, the use of negligible [1-^{13}$C]G-6-P should still be a good assumption to study glucose transport and metabolism in the heart too.

**DISCUSSION**

The results in Table 1 show that dynamic $^{13}$C NMR spectroscopy can directly provide the quantity $k_2 + k_3$ and thus the tissue glucose half-life time at hyperglycemia. It is important to realize that this parameter is independent of $C_{*P,eq}/C_{*E,eq}$ (Tables 1 and 2) and can be obtained from a simple exponential curve fit of the normalized experimental signal intensities. Thus the accuracy of this parameter depends only on the assumptions made in the derivation of the rate equations and on the experimental accuracy of the signal intensities. The quality of the NMR data (e.g., Fig. 2) and the very small standard deviation of only 8% indeed indicate good experimental accuracy, and the assumptions made in deriving the rate equations have also been well tested. First of all, our experiments (Fig. 2) show that plasma glucose is already constant at hyperglycemia within 0.5 to 2 min, whereas the first spectrum (average over the first 84 s) shows only a small change with respect to control. Thus the derivation based on a constant total blood glucose level during the experiment is well founded. Second, our extract data show convincingly that the concentration of phosphorylated glucose is negligible compared with total glucose, thereby validating the use of the simplified Eqs. 10-12 for total glucose. Third, the GC-MS data indicate that the unlabeled glucose concentration is stable during the duration of our experiments in the cat, supporting the initial assumptions in Eqs. 1 and 2, in which the nonlabeled glucose does not contribute to the change in total glucose. Fourth, the results in Table 1 show that the determined hyperglycemic $k_2 + k_3$ values are independent of the vascular fraction and therefore can be determined accurately from the experimental data without any assumptions. However, to determine $k_1$ it is necessary to assume a certain plasma-to-tissue glucose ratio, whereas determination of $CMR_{Glc}$ requires an additional assumption.

In Tables 1 and 2, we indicate that the $k_1$ values determined by the initial rate approach at vascular fractions 3–5% are equal (within SD) to the ones obtained from $k_2 + k_3$ and $C_{*P,eq}/C_{*E,eq}$ using Eq. 11. This confirms the accuracy of our model, especially the assumption of unidirectional metabolism. Because the initial rate approach is less accurate than the use of Eq. 11, we will use the $k_1$ values derived from $k_2 + k_3$ in our subsequent calculations. It is clear from Tables 1–3 and from Eq. 11 that the choice of $C_{*P,eq}/C_{*E,eq}$ will profoundly influence $k_1$. In addition, further analysis of the results
in terms of cerebral influx and utilization rates and the comparison of these rates with the literature are complicated by the fact that the rate constants $k_1 - k_2$ determined in dynamic uptake experiments are concentration dependent. This dependence is expressed in the relationship between Sokoloff's constants and the Michaelis-Menten parameters for the individual rate constants $k_1$ and $k_2$.

$$k_3 (\text{min}^{-1}) = \frac{T_{\text{max}} V_d}{C_P^{\text{tot}} + K_t} \quad \text{and} \quad k_2 (\text{min}^{-1}) = \frac{T_{\text{max}} V_d}{C_E^{\text{tot}} + K_t} \quad (19)$$

in which $T_{\text{max}}$ (in $\mu$mol·g$^{-1}$·min$^{-1}$) and $K_t$ (in mM) are the Michaelis-Menten maximum glucose transport rate and half-saturation constant, respectively. Notice that all concentrations are in millimolar units, which is required based on Eq. 1, in which the use of equal units for the rate constants (min$^{-1}$) corresponds to equal units for the concentrations. Combination of Eqs. 17 and 19 gives

$$T_{\text{max}}/\text{CMR}_{\text{Glc}} = \frac{(C_E^{\text{tot}} - C_E^{\text{eq}})K_t}{(C_P^{\text{tot}} - C_P^{\text{eq}}) + (k_2 + k_3)C_P^{\text{eq}} + K_t} \quad (20)$$

It is important to see the implication of this equation, namely that the $T_{\text{max}}$-to-$\text{CMR}_{\text{Glc}}$ ratio, $K_t$, or the $C_P^{\text{eq}}$-to-$C_E^{\text{eq}}$ ratio is concentration dependent. It seems unlikely that glucose transport parameters will change in acute hyperglycemia, and it has been documented that hexokinase is saturated [$K_{\text{base}} = 0.067$ mM (Ref. 16)] at a very low tissue glucose concentration, leading to a concentration-independent $\text{CMR}_{\text{Glc}}$. (10, 16, 20). Thus it is to be concluded that the $C_P^{\text{eq}}$-to-$C_E^{\text{eq}}$ ratio depends on the plasma glucose concentration. To determine all relevant parameters, we use the $C_P^{\text{eq}}$-to-$C_E^{\text{eq}}$ ratio at normoglycemia and a $K_t = 5 \pm 1$ mM to determine $T_{\text{max}}$, $\text{CMR}_{\text{Glc}}$ and the normoglycemic $\text{CMR}_{\text{Glc}}$ from our experimentally determined $k_2$ and $K_t$ value at $C_P^{\text{eq}} = 24.9$ mM.

<table>
<thead>
<tr>
<th>This Study</th>
<th>Radio-label$^b$ NMR$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1 = 5$ mM</td>
<td>$1.04 \pm 0.20$</td>
</tr>
<tr>
<td>$k_2 = 5$ mM</td>
<td>$0.207 \pm 0.009$</td>
</tr>
<tr>
<td>$k_3 = 5$ mM</td>
<td>$0.184 \pm 0.025$</td>
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<tr>
<td>$k_2 + k_3$</td>
<td>$0.391 \pm 0.035$</td>
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<tr>
<td>$t_c$, min</td>
<td>$1.77 \pm 0.18$</td>
</tr>
<tr>
<td>$\text{CMR}_{\text{Glc}}$, $\mu$mol·g$^{-1}$·min$^{-1}$</td>
<td>$0.463 \pm 0.042$</td>
</tr>
</tbody>
</table>

$^a$Maximum deviation based on SE in measured $k_2 + k_3$ value and assumed range for $K_t$. $^b$Average over literature on human studies published until 1991, reviewed by Gjedde (9). $^c$Based on 1 type of fitting from Gruetter et al. (12), see text. $^d$Assumed (see Data processing protocol). $^e$Calculated from reported $K_t$, $T_{\text{max}}$, and $\text{CMR}_{\text{Glc}}$ values. $^f$Estimated based on literature data. $^g$Reported value.

**Fig. 4.** High-resolution $^1$H spectra ($^1$H frequency 125 MHz; 11.7 T Magnet) of brain extracts collected in vivo (A, C) and postmortem (B, D). Spectra for a phantom with both [1-13C]glucose-6-P and [1-13C]glucose are shown in E. Spectrum of a heart extract (high-working heart preparation perfused with buffer containing [1-13C]glucose, [U-13C]palmitate, ketone bodies, Krebs solution, blood, and insulin) is given in F. Success of in vivo brain experiment is confirmed by low lactate level (resonance at 21 ppm). Standard is 1 mM acetate (at 24 ppm). Sample sizes and number of scans were 200 mg/3 ml tissue (20,000 scans), 1.6 g/3 ml tissue (10,000 scans), 1 mM (6,000 scans), and 2 g/3 ml (8,000 scans) for in vivo brain, postmortem brain, phantom, and heart, respectively. Data show negligible presence of [1-13C]glucose-6-P (resonances at 96.95 and 93.11 ppm for β- and α-isomers, respectively) in brain and only a small contribution (<4%) in heart. GABA, γ-aminobutyric acid; glu, glutamate; gln, glutamine.

**Fig. 5.** Plots of $T_{\text{max}}$ (A) and $\text{CMR}_{\text{Glc}}$ (B) at different $K_t$ values vs. plasma-to-tissue glucose equilibrium ratio at normoglycemia ($C_P^{\text{eq}} = 4$ mM). Functions describing relationships between these parameters are given in Eqs. 17, 19, and 22, and parameters are derived from experimentally determined $k_2 + k_3$ value at $C_P^{\text{eq}} = 24.9$ mM.
glycemic tissue-to-plasma glucose ratio on this determination is depicted in Fig. 5, A and B. Our numbers (Table 3) compare well with a recent literature compilation of gray matter radiolabel data by Gjedde (9) as well as with a recent compilation of static \(^{13}\)C and dynamic proton NMR transport studies from human NMR (12). To judge whether this is reasonable, we obtained MR images to determine the gray-to-white matter ratio in brain slices chosen for our cat studies. Figure 6 shows a coronal image with a typical slice position indicated. Using a voxel count of the gray and white matter regions, we determined that the gray-to-white matter ratio of the total area is \(80:20\%\). Because white matter has about one-half the aerobic metabolic rate (9) of gray matter and because rates are expected to be lower in anesthetized animals, it can be concluded that there is good agreement between our metabolic and transport data in an area of predominantly gray matter in the awake human. Actually, with the use of our experimentally determined \(k_2 + k_3\) value and the plausible \(K_t = 5 \pm 1\) mM, the range of possible \(T_{\max}\) and \(\text{CMR}_{\text{Gl}}\) values is limited (Fig. 5 and Table 3), because a reasonable corresponding \(C_{\text{tot}}^{\text{E}_{\text{eq}}}/C_{\text{E}_{\text{eq}}}^{\text{Toy}}\) value to exist (Eq. 20). With the use of our results, some insight can be obtained into recent data obtained by Gruetter et al. (12), who gave two potential combinations of \(K_t\), \(T_{\max}\), and \(\text{CMR}_{\text{Gl}}\) values, namely the ones in Table 3 and another combination, \(K_t = 4.8\) mM, \(T_{\max} = 0.80\) \(\mu\)mol \(g^{-1}\cdot\text{min}^{-1}\), and \(\text{CMR}_{\text{Gl}} = 0.32\) \(\mu\)mol \(g^{-1}\cdot\text{min}^{-1}\). However, this second series leads to a \(C_{\text{tot}}^{\text{E}_{\text{eq}}}/C_{\text{E}_{\text{eq}}}^{\text{Toy}}\) ratio of 14.4 and therefore can be discounted. We would also like to point out that, based on Eq. 19, \(T_{\max}\) can be determined within a small range by measuring \(C_{\text{tot}}^{\text{E}_{\text{eq}}}/C_{\text{E}_{\text{eq}}}^{\text{Toy}}\) and \(k_2 + k_3\) at hyperglycemia because the contribution of \(K_t\) is small in the denominator. To obtain an experimental value for \(K_t\), for more accurate determination of \(\text{CMR}_{\text{Gl}}\), the rate constants have to be studied as a function of concentration.

In summary, we have designed a new glucose-uptake NMR spectroscopy experiment to determine tissue glucose utilization half-life times from a simple exponential fit of the experimental signal intensities. It was demonstrated that this model simplification is allowed for the brain through NMR extract studies, which showed a negligible concentration of phosphorylated glucose compared with glucose. One organ that should also be suitable for this approach is the heart, in which the phosphorylated glucose concentration is \(<4\%\) of the total glucose concentration (Fig. 4F). The validity of this simplification for other tissues needs to be established using extract studies. The rate constants of brain glucose influx and utilization determined using this new approach are in the same order of magnitude as those obtained from previous PET and NMR studies, but the present approach is simpler. One important reason that the rates can be determined accurately is because the rate constants (not the rates) of transport and metabolism are slowed down at hyperglycemia. Actually, the principles established in this paper can also be applied to dynamic PET studies of radiolabeled \(^{11}\)C- or \(^{14}\)C-glucose. These studies are normally complicated by the fact that labeled metabolic products are impossible to distinguish from labeled glucose, necessitating the use of glucose analogs such as fluorodeoxyglucose. With the use of hyperglycemia (unlabeled glucose hyperglycemic steady state), it should be possible to slow down glucose utilization so that the glucose uptake curve reaches equilibrium before appreciable amounts of the first metabolic product (glutamate) are formed. The use of natural radioactively labeled glucose analogs and the possibility to measure half-life times with a single exponential data fit should allow accurate localized pathology studies using PET. Because PET studies use tracer concentrations, the labeled and unlabeled blood glucose concentrations can easily be kept stable and all rate equations derived here apply. Similar arguments hold for proton NMR, but, although metabolic products should be distinguishable, these measurements are complicated by the fact that difference spectroscopy has to be performed and that present sensitivity may be too low to perform time-resolved imaging studies. However, technical progress in NMR is ever continuing, and it may be possible that some of these problems can be solved in the near future.

We are grateful to Daryl DesPres for the animal preparations at the National Institutes of Health (NIH) and to Drs. M. Miyabe and J. Ulatowski for help with some of the experiments at Johns Hopkins. We also thank Dr. Richard Trastman for supporting this research, Drs. J. Ianhui Shi and J. Ack Cohen for helpful discussions, Alan Olsen and Dr. V. P. Chacko for technical support, and Drs. David Cohen and Maren Laughlin for helpful comments with the manuscript.

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