Test-Retest Studies of Cerebral Glucose Metabolism Using Fluorine-18 Deoxyglucose: Validation of Method

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In studies using [^{18}F]deoxyglucose (FDG), one often wants to compare metabolic rates following stimulation (drug or motor-sensory) with the baseline values. However, because of reproducibility problems with baseline variations of 25% in the same individual not uncommon, the global effect of the stimulation may be difficult to see. One approach to this problem is to perform the two studies sequentially. This means that, with the 110-min half-life of ^{18}F, one must take into account the residual activity from the first study when calculating metabolic rates for the second. We performed TEST-RETEST baseline studies on four subjects, with a 1-hr interval between injections. These studies were done without stimulation, in order to validate the repeatability of the method. To reduce the amount of residual activity from the first study, the first injection was only 2 mCi in three cases, and only 1 mCi in one case, out of a total injected dose of 5 mCi. A correction for residual activity was included in the RETEST calculation of metabolic rate. The results showed a global metabolic shift between the two studies of 2% to 9%. An error analysis shows that the shift could be further reduced if anatomically comparable scans are done at comparable postinjection times.

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Positron emission tomography (PET) scanning with fluorine-18 deoxyglucose (FDG) is now generally accepted as a method for measuring glucose metabolism in the brain. In many studies it is desirable to measure glucose utilization under two different conditions, such as a baseline or unstimulated state followed by a period of stimulation with drugs, physical activity, or mental tasks. However, even intra-subject baseline metabolic rates can vary considerably from day to day, and performing studies on separate days is logistically more difficult. Therefore it would be desirable to perform the two studies sequentially on the same day, as has been done at other centers with carbon-11 (^{11}C) deoxyglucose, taking advantage of its short, 20-min half-life (*2*).

To perform TEST-RETEST studies with [^{18}F]deoxyglucose, which has a 110-min half-life, a method must be found to handle the residual activity trapped during the TEST study which will still be present when the RETEST scans are done. We have developed two approaches to counteract this problem. First, we give a larger dose in the second study; second, we apply a mathematic correction for the residual activity from the TEST study, based on nominal rate constants for FDG. The injection and scanning strategy is illustrated in Figure 1. In this particular implementation, two interleaved scans of seven slices each are done for both TEST and RETEST studies, yielding a total of 14 images per study.

In this paper we present the theory for performing the residual correction, including an analysis of the resulting error. We also give experimental results for four subjects who were maintained in a baseline condition throughout both studies. These studies were done to determine how much variation takes place between TEST and RETEST studies *without* stimulation, to establish a baseline repeatability (*1*).

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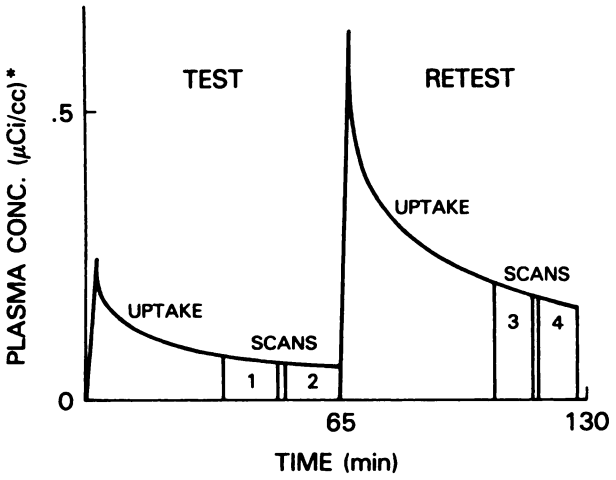
METHODS

Theory

The equations needed for the TEST-RETEST studies are based on the Sokoloff model (*3*), illustrated in Figure 2. Our



*decay corrected to t = 0

FIGURE 1
Typical curve of plasma radioactivity concentration versus time for a 2:3 injection ratio. The two peaks correspond to two injections. Two TEST scans (1 and 2) and two RE-TEST scans (3 and 4) are shown, beginning ~35 min after the respective injections. The couch position was incremented 6 mm between scans 1 and 2, and between scans 3 and 4 to interleave the resulting images.

starting point is the general equation for tissue concentration of tracer at time t (*4*):

$$c_i(t) = AI(0, t, \alpha_1) + BI(0, t, \alpha_2), \quad (1)$$

where $I(t_a, t_b, \alpha) = \int_{t_a}^{t_b} e^{-\alpha(t_b - t')} c_p(t')\, dt'.$

$c_p(t')$ is the plasma concentration of tracer at time t', and A, B, α_1, and α_2 are combinations of the rate constants $k_1 - k_4$ (Fig. 2), defined by

$$A = k_1(k_3 + k_4 - \alpha_1)/(\alpha_2 - \alpha_1)$$

$$B = k_1(\alpha_2 - k_3 - k_4)/(\alpha_2 - \alpha_1)$$

$$\alpha_{1,2} = \tfrac{1}{2}[k_2 + k_3 + k_4 \mp \sqrt{(k_2 + k_3 + k_4)^2 - 4k_2k_4}].$$

The interpretation of Eq. (1) is seen more easily if we note that generally $k_4 \ll k_2 + k_3$, so that

$$A \cong k_1k_3/(k_2 + k_3),$$

$$B \cong k_1k_2/(k_2 + k_3),$$

$$\alpha_1 \cong k_4,$$

$$\alpha_1 \cong k_2 + k_3.$$

In Eq. (1) and subsequent equations, all activity concentrations have been corrected for the physical decay of ^{18}F to a standard reference time.

Equation (1) states that there are two subpopulations of tracer in the tissue at time t: a long-lived component (half-life = (ln 2)/α_1, or ~2 hr) corresponding to FDG-6 phosphate plus free FDG that will be phosphorylated before it can return to the blood, and a short-lived component (half-life = (ln 2)/α_2, or ~3 min) corresponding to free FDG molecules that will return to the blood without being phosphorylated. This latter component is represented by open circles in Figure 2. (Of course one cannot predict the fate of an individual molecule, but one can predict the number of molecules which will undergo each fate.) We refer to the first population as "trapped," even though some molecules (the solid circles in the free compartment of Figure 2) have not yet been phosphorylated, and to the second population as the "washout" component because they will be washed out of the tissue by the blood before phosphorylation can take place. Note that the washout component is not equivalent to the total free tracer in the tissue.

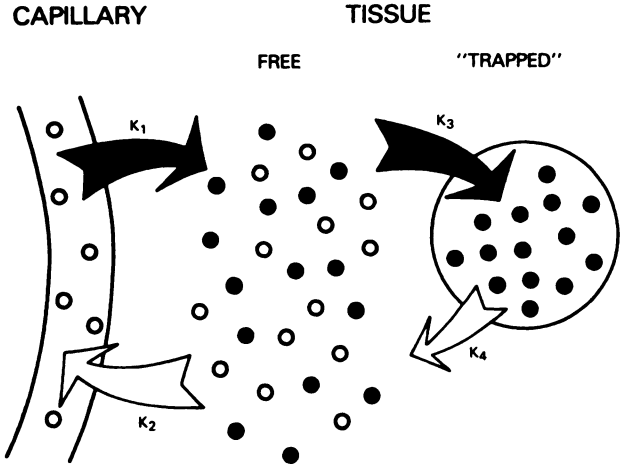


FIGURE 2
Sokoloff's three-compartment model for deoxyglucose. Compartment 1 is the capillary, compartment 2 is free FDG in tissue, and compartment 3 is phosphorylated, or trapped, FDG in tissue. The open circles are "visiting" FDG molecules which return to blood stream without being phosphorylated. Rate constants $k_1 - k_4$ are defined as the unidirectional mass transport rate of tracer across the (physical or chemical) boundary separating compartments, divided by tracer concentration on the driving side.

By combining Eq. (1) with the basic equation for glucose utilization rate (*4*):

$$R = \frac{c_g}{LC}\frac{k_1k_3}{k_2 + k_3}, \quad (2)$$

where c_g is the plasma concentration of glucose and LC is Sokoloff's lumped constant that reflects the kinetic differences between glucose and FDG, we obtain one form of the "operational" equation for glucose utilization (*5*):

$$R_{TEST} = \frac{c_g}{LC}\frac{c_i(t_1) - \overbrace{BI(0, t_1, \alpha_2)}^{\text{washout corr.}}}{\underbrace{\beta I(0, t_1, \alpha_1)}_{\text{phosphatase corr.}}}. \quad (3)$$

In this equation, β is a constant defined in Ref. (*5*) that is almost unity, and reflects the small difference between A and the factor $k_1k_3/(k_2 + k_3)$ that appears in Eq. (2).

Equation (3) is an alternative to Sokoloff's original operational equation (*3*). The main difference is that Sokoloff's equation has a correction term in the numerator which represents the *total* precursor, or unphosphorylated, tracer in the tissue, and a second correction term in the denominator

which, according to Sokoloff, corrects for the lag in tissue equilibration with plasma. In Eq. (3), these two terms are combined into a single correction which represents only those FDG molecules which return to the blood stream without undergoing phosphorylation, i.e., the short-lived, or washout, component of Eq. (1). Eq. (3) also incorporates a particularly simple phosphatase, or k_4, correction—it is merely the inclusion of an exponential factor containing α_1 in the integral in the denominator.

Equation (3) can be used as it stands for the first, or TEST, study. For the RETEST study, a correction term must be added for the residual activity left from the first study. The appropriate equation is as follows:

$$R_{RETEST} = \frac{\frac{c_g}{LC}\left[c_i(t_2) - BI(0, t_2, \alpha_2) - \overbrace{e^{-\alpha_1(t_2-t_1)}[c_i(t_1) - BI(0, t_1, \alpha_2)]}^{\text{residual corr.}}\right]}{\beta I(t_1, t_2, \alpha_1)}, \quad (4)$$

where t_2 is the time interval from the first injection to the RETEST scan and $c_i(t_2)$ is the tissue concentration of tracer at the time of the second scan.

The washout and phosphatase corrections are the same as before. Note, in particular, that the washout integral $I(0, t_2, \alpha_2)$ includes the entire blood activity curve from time 0 to t_2, although the contribution before t_1 is very small and could be neglected. The term labeled residual correction can be explained as follows: We start with the total tissue concentration of activity measured during the TEST scan, $c_i(t_1)$. We then subtract the washout subpopulation at that time, $BI(0, t_1, \alpha_2)$, because this component has a lifetime of only ~3 min and so has cleared the tissue by the time of the RETEST scan. The result is then multiplied by the factor $\exp(-\alpha_1(t_2 - t_1))$ which describes the exponential loss of the long-lived component (cf. Eq. 1). Note also that the plasma integral in the denominator of Eq. (4) extends only from t_1 to t_2, since the numerator has been modified to give only that part of the uptake that takes place between t_1 and t_2.

While we have focused on giving a physical interpretation of the RETEST equation, the reader of course realizes that this equation can be derived directly from Eq. (1), and that its validity does not rest solely on the above physical description. While the derivation depends on the rate constants being constant throughout the entire study, the question may arise whether the equation can accommodate to different rate constants during the TEST and RETEST phases. Indeed, if stimulation is applied, some rate constants must change, even if they started out as nominal. The question is largely academic, since the actual rate constants are generally not known and nominal values must be used. Nevertheless it is worth noting that Eq. (4) could be adapted, at least approximately, to permit the use of different rate constants for the TEST and RETEST phase, if they could be determined. Specifically, all terms but one refer to the RETEST period, following the second injection, so RETEST values of $k_1 - k_4$ are appropriate. The exception is the last term in the numerator, $BI(0, t_1, \alpha_2)$, which represents the washout component during the TEST scan, and which therefore would ideally be calculated using rate constants appropriate to the TEST period.

The following is an examination of the sensitivity of the residual correction term to deviations of the rate constants from their nominal values, assuming that the same rate constants are used throughout the calculation. Blood curves and typical tissue concentrations from two of the studies reported herein will be used. One calculation is for a 2:3 dose ratio study and a tissue region with a nominal glucose utilization of 11.1 mg/100 cc/min; the other calculation uses a 1:4 ratio study and a region with 7.4 mg Glu/100 cc/min nominal utilization. Since the effect depends on the exact blood curve and tissue concentrations, these calculations are meant to suggest the general range of error, and not to be a definitive error calculation. In each case the residual correction term was calculated first using nominal rate constants from Ref. (*4*), viz., $k_1 = 0.102$, $k_2 = 0.13$, $k_3 = 0.062$, and $k_4 = 0.0068$ min^{-1}, and then with values ranging from one-half to twice nominal. Because k_1 and k_2 refer to the same physical transport mechanism, we assumed, as an approximation, that their variations are coupled, i.e., k_1/k_2 = constant. In these calculations we compared scans 1 and 4, in order to maximize the time difference between scans.

The first point of interest is that the magnitude of the residual correction terms was 15–20% of the total numerator for the 2:3 dose ratio, and 5–10% for the 1:4 ratio. This means it is comparable in magnitude to both the washout and phosphatase corrections. A second point of interest is that the k's appear only to second order in this term, compared to the leading term $c_i(t_1)$, so we expect it to be less affected by variations in the k's.

Figure 3 shows the change in the correction term when the rate constants deviate from nominal, divided by the total numerator of Eq. (4) (except for c_g) and multiplied by 100. In other words, these plots show the percent change in metabolic rate caused only by the effect of k variations on the residual correction term. As expected, this error is not very great, being <4% over most of the variation range. While this calculation is only suggestive, since it is based on particular cases, it is clear that the residual correction is robust in its insensitivity to changes in the rate constants.

Of course it would be unrealistic to assume that the use of incorrect nominal k's affects only the residual correction term. It also affects the washout and phosphatase corrections, and the effect on all these terms could be coupled. Therefore we calculated the metabolic rates for the same regions as above, but we allowed the k's to vary in all terms. In this case we focused on the relative change between TEST and RETEST scans and how it is affected by using different rate constants. That is, we calculated R_{RETEST}/R_{TEST}, using both nominal rate constants and deviate ones. The difference between the two results, multiplied by 100, is the % change between TEST and RETEST caused by using wrong rate constants, and so is a measure of the % error. Thus, by focusing on the change between TEST and RETEST, we eliminate the common effect of k variations on both measurements, which are not of interest at this time.

An important result came out of this analysis. We found that the percent error in $R_{RETEST} - R_{TEST}$ was minimized when scans taken at the same relative times after the respective injections were compared. Specifically, the error was less comparing scans 1 and 3 or scans 2 and 4, than when comparing scans 1 and 4 or scans 2 and 3. The reason for this is clear: the effect of k-variations on the washout and phosphatase corrections are approximately equal when both studies are done at the same relative postinjection time, so there is

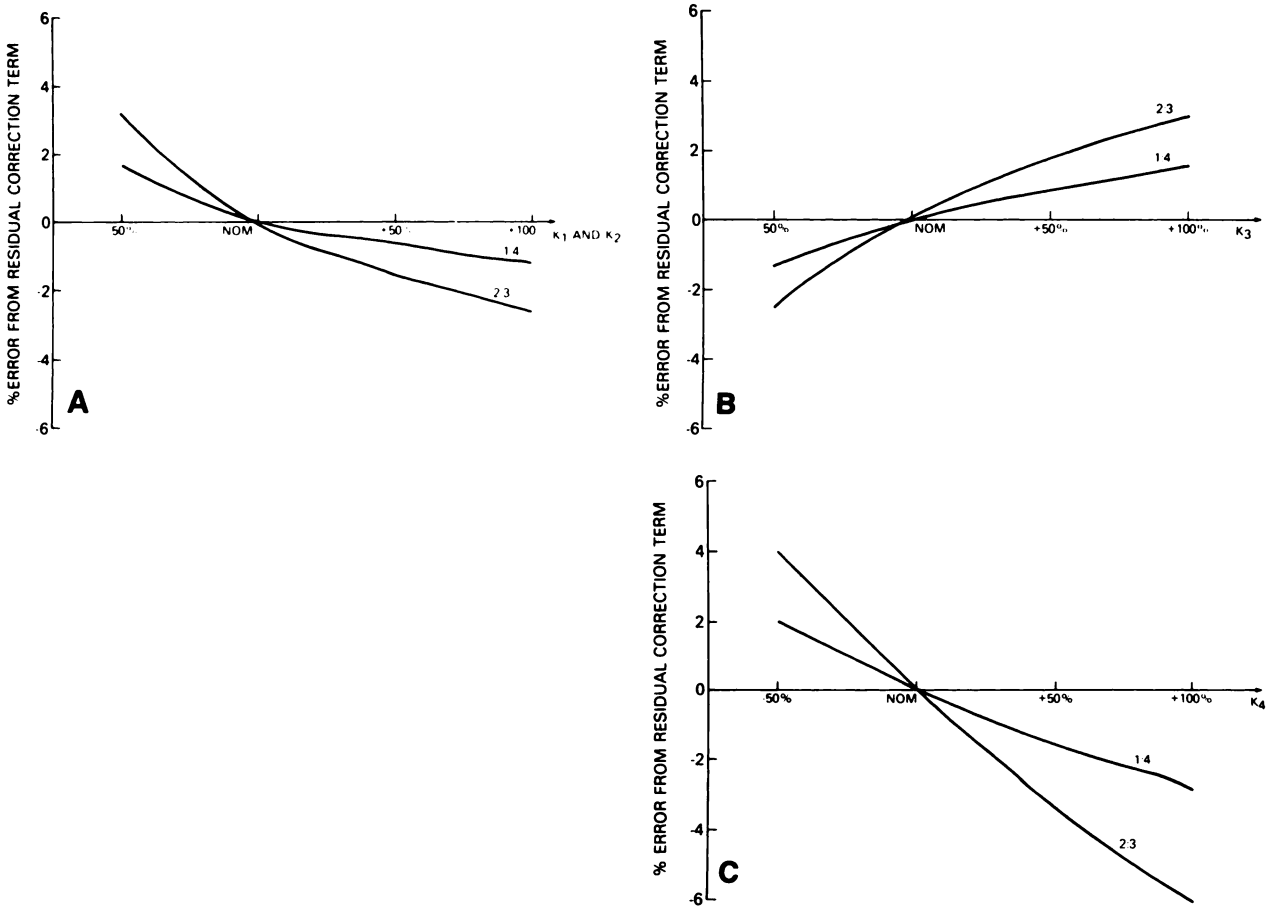


FIGURE 3
Percent error in metabolic rate in the RETEST study caused by allowing the rate constants to deviate from their nominal value in the residual correction term only. (A) $k_1 - k_2$ deviation. (B) k_3 deviation. (C) k_4 deviation.

less artifactual difference between studies due to errors in the rate constants. Therefore ideally the TEST and RETEST scans at the same anatomical levels should be isochronal, i.e., done at the same post-injection items. Figure 4 shows a typical error plot when scans 1 and 3 are compared; the results for scans 2 and 4 are similar. We see that the errors are within ± 4% over most of the range of variation, which is the same order of magnitude as the error in the residual correction alone.

Patient Studies

TEST-RETEST studies were performed on four subjects: two normal volunteers and two patients with brain tumor. All scanning was done with the Neuro-PET scanner (*6*). The allowed patient dose of 5 mCi of FDG was divided into two aliquots. In three cases 2 mCi were administered intravenously for the TEST scans and 3 mCi for the RETEST scans. In one case the dose division was 1 and 4 mCi. Activity in the blood was monitored by taking blood samples at frequent intervals from a vein in the contralateral heated hand. A typical plasma activity curve is shown in Figure 1.

After allowing 35–40 min for trapping, scans 1 and 2 were performed. Each scan provides seven images, or slices, with a center-to-center spacing of 11.5 mm. The patient couch was incremented 6 mm between the two scans, so that the result was 14 interleaved images with ~6 mm center-to-center spacing. Scan times were 15 min, longer than the usual 10 min in order to compensate partially for the smaller dose. After completion of the first two scans the second dose of FDG was injected. Since the object was to examine baseline reproducibility, no stimulation was applied and the subject remained in the scanner throughout. After another 35–40 min for trapping, scans 3 and 4 were performed with a scan time of 10 min each. The couch was not moved between scans 2 and 3, but was incremented after scan 3 to return it to the original position for scan 4.

Measurements of activity concentrations were made from the images using the Neuro-PET region-of-interest program. Global measurements were taken as the average value for a large ellipse encompassing the entire brain. Local cortical readings were taken from selected images using the Neuro-PET cortical map program that places 32 regions, in this case 13 mm diameter circles, at equal angular increments around the brain. These circles are automatically moved radially inward until the average reading for each circular region is maximized. Metabolic rates were calculated using Eqs. (3) and (4), as appropriate. RETEST rates were calculated using residual concentrations from corresponding regions in the anatomically comparable TEST scan. Because of the positioning sequence used, scan 1 was used to correct scan 4, and scan 2

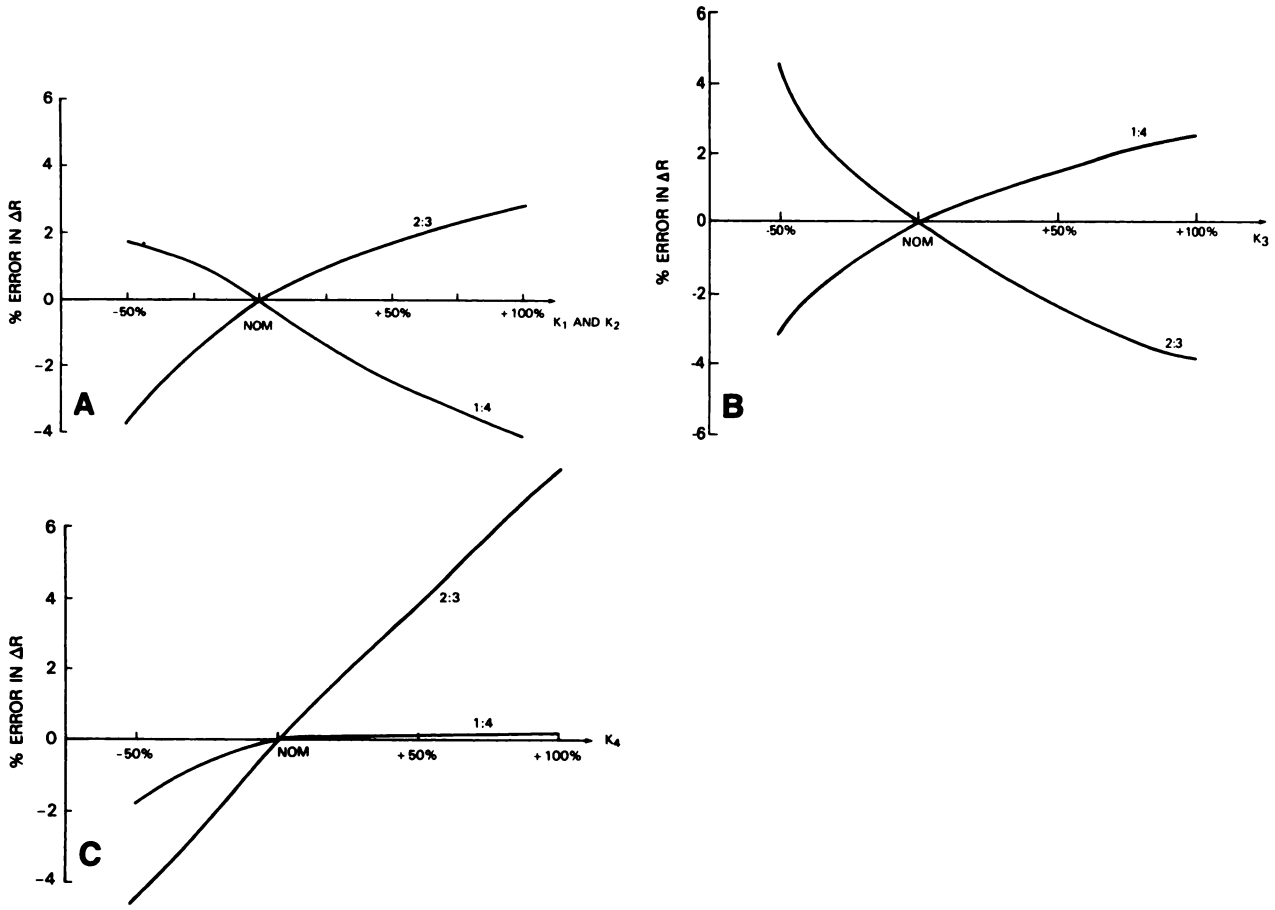


FIGURE 4
Net error in $\Delta R = R_{RETEST} - R_{TEST}$ when the rate constants deviate from nominal in all terms, expressed as a percent of R_{TEST}. (A) $k_1 - k_2$ deviation. (B) k_3 deviation. (C) k_4 deviation.

to correct scan 3. Nominal rate constants were used throughout, as previously indicated, and the lumped constant was 0.42 (*3*).

RESULTS

Typical images from two studies are shown in Figure 5. The color scale has been adjusted in each pair of images to encompass exactly the same range of metabolic rates, so that numerical comparisons can easily be made. Since the ability to do the RETEST calculation pixel by pixel does not exist in the Neuro-PET, the RETEST images do not include the residual correction, but are actually a weighted average of both TEST and RETEST uptakes. The pictures are nevertheless useful since the dose ratio, plus the physical decay of ^{18}F, makes them approximately equivalent to corrected images. Note that in the study with a 1:4 dose ratio, the patient inadvertently moved between scans 2 and 3; fortunately the motion was very close to the slice spacing, so that the same scan comparisons were made, except that slice 7 of the TEST scans was compared with slice 6 of the RETEST ones, etc.

The predominant difference between TEST and RETEST scans was a global shift in metabolic rate. By averaging all global metabolic rates for images that showed complete and comparable brain sections, we found that the RETEST metabolic rates were 9% and 5% higher for the two normal volunteers, 2% higher for one tumor patient, and 7% lower for the other. These changes can be appreciated visually by carefully examining the colors in Figure 5.

A study of the images also indicates that no significant local variations, beyond the global shift, are present. This is confirmed by the local cortical map calculations. For example, for the first three studies, excluding the one with a position shift, the standard deviation of the percent TEST-RETEST differences for the 32 cortical regions was 5.4%, 5.6%, and 7.7%, which is not much different from statistical errors (see Discussion).

DISCUSSION

Besides the usual sources of error in PET scans, special attention must be paid to the following error

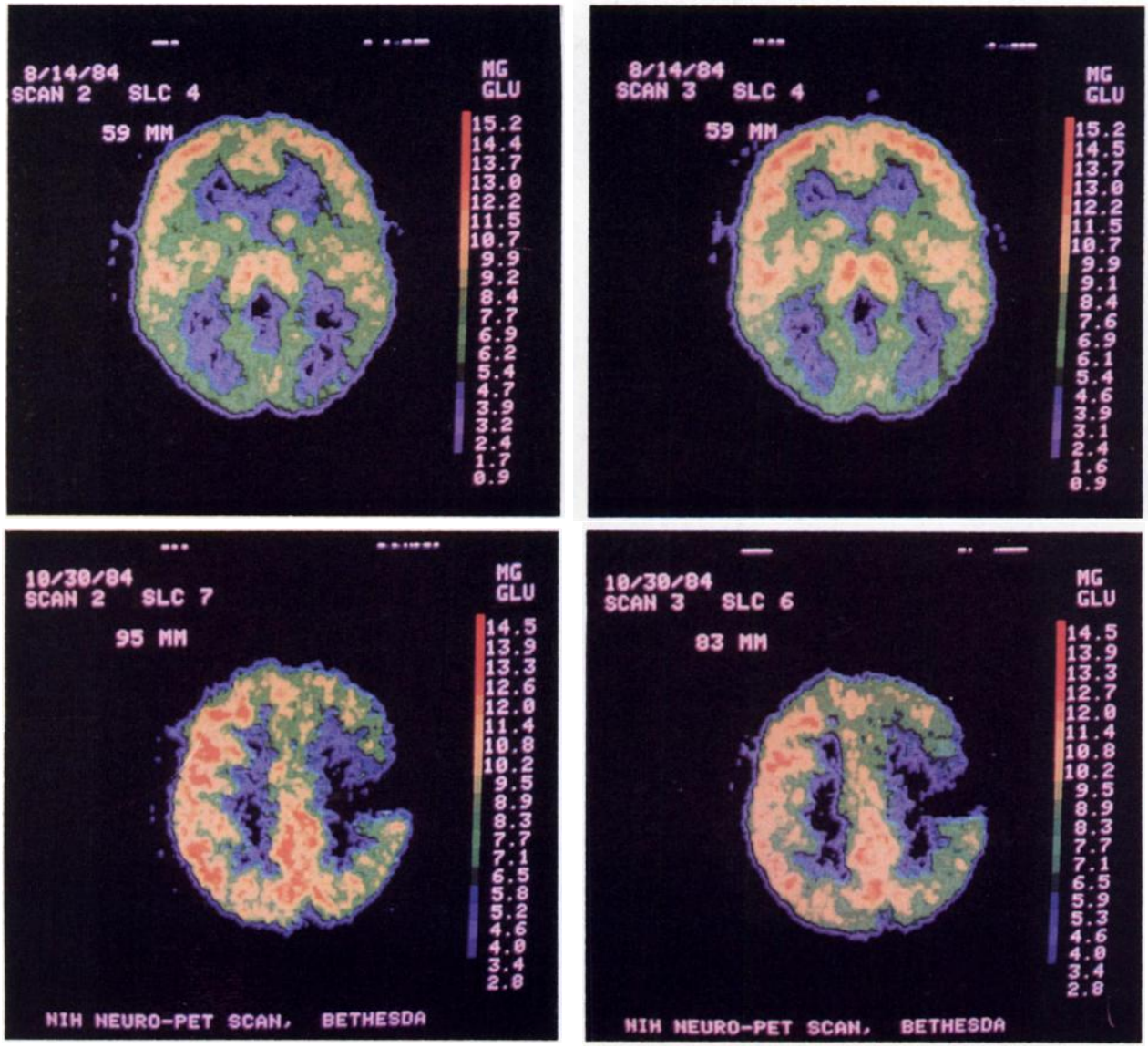


FIGURE 5
Typical TEST (left) and RETEST (right) images for a normal volunteer (A) and a tumor patient (B). The color scales are adjusted in each pair of images to represent the same range of metabolic rates.

sources for TEST-RETEST comparisons: (a) anatomic position errors, (b) relative errors in the washout and phosphatase corrections, (c) error in the residual correction, (d) statistical error due to low counts, particularly in the TEST scan, and (e) count rate nonlinearity of the scanner. These are discussed separately below.

It is obvious that reproducibility of patient position is of critical importance when measuring changes in metabolism for small brain structures. A possible advantage of sequential TEST-RETEST scanning is that the subject can remain in the scanner throughout the procedure, if this is consistent with the type of stimulation used. With a strongly restraining head holder, this is probably the best method of ensuring accurate positioning. Alternatively, one can realign the subject's position before performing the RETEST scans. Because the head holder on the Neuro-PET is not strongly restraining, it is not surprising that in one study the patient's position slipped between the TEST and RETEST studies.

The error analysis reported earlier indicates that errors in the standard washout and phosphatase corrections can be held to near-negligible levels if the scans being compared are isochronal; i.e., performed at the same postinjection time interval. This observation applies as well to comparison scans done on different days. The isochronal strategy was not incorporated in the present studies because the point was only appreciated after the analysis had been performed. It is likely, therefore, that some of the global shift we observed is

due to differences in the standard FDG corrections caused by deviations of the rate constants from their nominal values.

The source of error that is unique to the sequential TEST-RETEST studies is the residual correction term. Our error analysis suggests that the error in this term caused by deviations of rate constants from the nominal values is <4%. It is worth noting, however, that this error depends on the time interval between TEST and RETEST scans. With longer times, the magnitude of the term declines because of decay and washout, but also the error caused by uncertainty in k_4 increases. A simple analysis of k_4 dependence shows that the error starts at zero for zero time difference (because the uncertainty in the k_4 correction is zero), peaks at around 80 min, and then declines toward zero again as the magnitude of the term gets smaller, i.e., as the two studies become independent. While it is tempting to make the interstudy time longer or shorter, to reduce this error, it is probably not practical because of considerations of patient handling and stimulation time.

The question of statistical error is particularly relevant because of the smaller dose used in the first study. This means that the TEST scans are noisier, and the statistical error for measurements in small regions of interest will be larger. We were able to overcome this problem with the 2:3 dose ratio by elongating the TEST scans to 15 min, so that both sets of scans contained about the same number of counts. However, with the 1:4 ratio there still remains a shortage of counts in the TEST scans. Let us compare the resulting statistical uncertainty with that of a standard 10-min scan following equal dose division, i.e., a 2.5-mCi injection. Such images on the Neuro-PET contain ~3–4 million counts and measurements on brain-sized phantom images with 3 million counts show a pixel-to-pixel standard deviation of ~20%. Using the square root of N law (which is only approximately valid for reconstructed images), we find that the standard error of the mean for a region of interest containing 25 pixels, e.g., a 12 mm circle, is ~4%. If the dose is reduced to 1 mCi and the scan time increased to 15 min, we would collect only 60% of these counts, which leads to an s.e.m. ~30% higher, or 5.2% for the TEST images. On the other hand, there will be more counts in the RETEST images, because of the greater dose. A simple calculation shows that the net error in the TEST-RETEST difference due to count-limited statistics will increase from 5.6% with equal dose division to 6.2% with the 1:4 dose ratio as described.

With higher count rates in the RETEST scans, scanner count-rate linearity becomes important. Measurements of scanner calibration at different count rates should be made, and correction factors inserted if necessary. Measurements of count-rate linearity on the Neuro-PET subsequent to the TEST-RETEST studies have resulted in a modification of the linearity corrections, and suggest that some undetermined part of the shift we observed was due to count rate effects.

Finally, we should mention the possible effect of isotope contamination. It is now recognized that the method of preparing FDG that we and others employ leads to a significant mannose contaminant (*7*). Spot checks have shown that the impurity level is 10–15% for the NIH production. While this will clearly have an effect on calculated metabolic rates, the point to be made here is that it does not affect the residual correction term, since the disappearance of the residual activity follows the empirical rate constants determined from dynamic studies, using impurity-containing FDG, which is valid regardless of the chemical form of the activity.

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