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The Lumped Constant in the Deoxyglucose Procedure Declines with Age in Fischer-344 Rats

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Abstract: Concentrations of [^{14}C]2-deoxy-D-glucose ([^{14}C]DG) and of glucose were measured in plasma of arterial and sagittal sinus venous blood from awake Fischer-344 rats at 3, 12, and 24 months of age, during continuous intravenous infusion of [^{14}C]DG and after a steady-state arterial plasma concentration of [^{14}C]DG was reached. Brain extraction, i.e., the difference between arterial and venous plasma concentrations divided by the arterial plasma concentration, was calculated for both [^{14}C]DG and glucose. Because exchange of both substances between rat plasma and erythrocytes is slow, the ratio of the brain extraction of [^{14}C]DG to that of glucose is identical to the lumped constant in the deoxyglucose procedure of Sokoloff et al. [*J. Neurochem.* **28**, 897–916. (1977)]. This ratio equaled 0.502 ± 0.015 (SEM) at 3 months, 0.456 ± 0.007 at 12 months, and 0.418 ± 0.006 at 24 months of age (n = 15); the means differed significantly from each other ($p < 0.05$). The results indicate that the lumped constant declines between 3 and 24 months of age in awake rats, and suggest that many reported age reductions in regional cerebral glucose utilization, of 15–25%, are artifactual. **Key Words:** [^{14}C]2-Deoxy-D-glucose—Glucose—Brain—Oxidative metabolism—Aging—Rat—Lumped constant. **Takei H. et al.** The lumped constant in the deoxyglucose procedure declines with age in Fischer-344 rats. *J. Neurochem.* **46**, 931–938 (1986).

It has been demonstrated with the [^{14}C]2-deoxy-D-glucose ([^{14}C]DG) technique of Sokoloff et al. (1977) that the regional cerebral metabolic rate for glucose ($rCMR_{glc}$) declines between 4–6 months and 14–16 months of age in many brain regions of awake Sprague-Dawley rats (Smith et al., 1980), and between 3 and 12 months in awake Fischer-344 rats (London et al., 1981). However, regional cerebral blood flow (rCBF), as measured with [^{14}C]iodoantipyrine, does not fall significantly between 3 and 12 months of age in awake Fischer-344 rats, and does not decline in most supratentorial cortical regions even after 24 months (Ohata et al., 1981). Fischer-344 rats have a maximum life span of about 35 months and a mean survival time of 29 months (Coleman et al., 1977).

rCBF is coupled to brain oxidative metabolism under normal conditions and in chronic pathological states (Raichle et al., 1976; Sokoloff, 1978). Coupling accounts for the observed proportionality between rCBF and $rCMR_{glc}$ in individual brain regions of awake and anesthetized animals (Reivich, 1974; Sokoloff, 1978). Therefore, the different courses of $rCMR_{glc}$ and of rCBF during aging of the rat could reflect altered coupling relations between flow and oxidative metabolism. A change in coupling has been described, for example, during maturation of the sheep brain (Jones et al., 1982).

On the other hand, some reported age declines of $rCMR_{glc}$ in the rat might be artifactual, if they reflected an age reduction in the "lumped constant" that is used to calculate $rCMR_{glc}$ from the brain uptakes of intravenously injected [^{14}C]DG and glucose (Sokoloff et al., 1977). The latter interpretation is suggested by observations that global CBF and the directly determined global cerebral metabolic rates for glucose (CMR_{glc}) and for O_2 ($CMRO_2$) do not differ significantly between 3 and 24 months of age in awake Fischer-344 rats (Takei et al., 1983).

We therefore decided to determine the lumped

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Abbreviations used: CMR_{glc} $CMRO_2$, cerebral metabolic rates for glucose and oxygen, respectively; CV, coefficient of variation; DG, 2-deoxy-D-glucose; rCBF, regional cerebral blood flow; $rCMR_{glc}$, regional cerebral metabolic rate for glucose.

constant in relation to aging of awake Fischer-344 rats, by measuring extractions by brain of [^{14}C]DG and of glucose from cerebral arterial blood, once steady-state plasma concentrations were established for both substances. At a steady state, net extraction of either substance equals the quantity that enters the brain metabolic pool. Monosaccharide exchange between red blood cells and plasma of rat whole blood is so slow (Heath and Rose, 1969; Wagner et al., 1984), furthermore, that arterial and venous plasma rather than whole blood concentrations of [^{14}C]DG and of glucose can be used to calculate the lumped constant.

MATERIALS AND METHODS

Seventy-eight male Fischer-344 rats, aged 3, 12, or 24 months (Charles River Breeding Laboratories, Wilmington, MA, U.S.A.) were studied within 2 weeks of their delivery to the laboratory. The surgical procedure employed has been described in detail by Takei et al. (1983). Surgery was performed in two stages. During the evening prior to an experiment, a rat was anesthetized by methoxyfluorane inhalation (Metofane, Pitman-Moore, Washington Crossing, NJ, U.S.A.). The scalp was incised longitudinally, and a 1-mm diameter burr hole was made in the skull above the caudal end of the superior sagittal sinus (Zeman and Innes, 1963). Continued bleeding from the skin edge and galea was prevented by electrocoagulation. An autoclip was fixed with cyanoacrylate (Super Glue, Ridgewood, NY, U.S.A.) on the exposed skull surface adjacent to the burr hole, as a stay for the needle that was to be inserted later. The skin was clamped, and the rat was allowed to recover from anesthesia in a cage where it had free access to water but not to food.

The following morning, the rat was reanesthetized with methoxyfluorane, and 10-cm long polyethylene catheters (PE 50) that were filled with 100 IU of Na heparin/ml of Normosol-R-pH 7.4 (Replacement Electrolyte, Abbott Laboratories, N. Chicago, IL, U.S.A.) were tied into the right femoral artery and vein. The skin was sutured at the catheter exits and infiltrated with bupivacaine HCl (Marcaine, Breon Laboratories, New York, NY, U.S.A.). A 21-gauge needle, with the tip filed to reduce the taper and cutting edge, was inserted about 0.6 mm into the superior sagittal sinus in a caudal direction, with the edge extending to the torcula, and glued with cyanoacrylate to the skull and external stay. The needle was connected to silastic medical grade tubing, 0.63 mm internal diameter (Dow Corning, Midland, MI, U.S.A.), which was filled with heparinized Normosol-R-pH 7.4. Hemostasis was accomplished by applying oxidized regenerated cellulose (Surgicel, Johnson and Johnson, New Brunswick, NJ, U.S.A.) to the area.

Following surgery, the hindquarters of the rat were wrapped in a fast-setting plaster bandage (Johnson and Johnson), which was taped to a lead block. Both forelimbs were tied loosely to the block to prevent pulling on the tubing that was connected to the sagittal sinus needle. The rat was allowed to recover from anesthesia for at least 4 h in a sound-dampening chamber (London et al., 1981). Body temperature was maintained at 36°C by connecting a rectal thermoprobe to a temperature feedback device that could activate heating wires on the roof of the chamber. Arterial blood pressure and pulse rate were recorded from the arterial catheter by means of a strain gauge transducer (Statham Instruments, Oxnard, CA, U.S.A.).

Nine rats, in addition to those noted above—three at 3 months of age, three at 12 months, and three at 24 months—were anesthetized and had indwelling catheters placed in their femoral artery and vein, and then were allowed to recover for 4 h. Each rat was injected intravenously with 50 μCi of [^{14}C]DG (sp act 56 mCi/mmol; New England Nuclear, Boston, MA, U.S.A.). Samples of femoral artery blood were removed periodically for up to 45 min and centrifuged, after which plasma aliquots were removed and their radioactivity was determined by liquid scintillation spectroscopy after addition of 10 ml of Ready-Solv, a scintillation cocktail (Beckman Instruments, Irvine, CA, U.S.A.).

Measured arterial plasma radioactivities were fit by nonlinear least-squares to a sum of exponential terms, which then were subjected to impulse analysis with Laplace transform techniques to calculate infusion schedules that would produce approximately constant plasma [^{14}C]DG concentrations in rats at 3, 12, or 24 months of age (Patlak and Pettigrew, 1976; see Acknowledgment). Each infusion rate could be represented by the following equation, where A, B, and D are constants in the units of ml · min^{-1}, t is in min, and α and β are constants in units of min^{-1}:

$$\text{Infusion rate} = A\exp(-\alpha t) + B\exp(-\beta t) + D \quad (1)$$

It was determined that A = 0.077, 0.0421, and 0.0506 for rats at 3, 12, and 24 months, respectively; B = 0.114, 0.336, and 0.354; D = 0.0279, 0.0278, and 0.0241; α = 0.130, 0.104, and 0.097; β = 0.858, 1.486, and 1.171. In addition, a priming injection of 0.023 ml, 0.127 ml, or of 0.108 ml at 3, 12, or 24 months, respectively, was administered prior to infusion.

From the 78 rats with indwelling catheters in the sagittal sinus, which had recovered from anesthesia for at least 4 h, 20-μl samples of femoral artery blood were collected prior to and at the end of [^{14}C]DG infusion so as to determine "initial" and "final" hematocrits (Micro-Hematocrit Reader, No. 1000, Clay-Adams, Parsippany, NJ, U.S.A.). In addition, 0.2 ml of arterial blood was removed anaerobically in a heparinized glass syringe, prior to [^{14}C]DG infusion, or analysis of P_aO_2, P_aCO_2, and pH (pH-Blood Gas Analyzer, No. 213, Instrumentation Laboratories, Lexington, MA, U.S.A.). A 50-μl arterial sample also was collected initially and was centrifuged, for the determination of plasma glucose concentration (Glucose Analyzer II, Beckman).

Following these procedures, the rat was administered a loading injection of [^{14}C]DG (see above), and then was infused intravenously for 40 min, at rates given by Eq. 1, with a solution of 15 μCi of [^{14}C]DG/ml isotonic saline. Infusate was delivered by a syringe pump (Model 352, Sage Instruments, Orion Research, Boston, MA, U.S.A.), at a flow rate controlled by a programmed microprocessor.

Periodically during infusion, paired 120-μl samples of blood from the femoral artery and superior sagittal sinus were collected in precooled vials. Centrifugation was initiated within 10 s and completed within 55 s after removal of blood. Plasma aliquots were withdrawn and were analyzed in duplicate for glucose (see above), and in trip-

licate for radioactivity by means of liquid scintillation spectroscopy (Model LS 9000 Liquid Scintillation Spectrometer, Beckman).

Exchange of [^{14}C]DG and glucose between red blood cells and plasma in rat whole blood

In a series of in vitro experiments, 6 ml of blood was removed by intracardiac puncture from 3-month-old rats, into a syringe containing 1,000 IU Na heparin (0.1 ml). The blood and heparin were mixed gently and the contents of the syringe were placed in a vial at room temperature or at 37°C in a water bath. A 200-μl sample of blood was taken and centrifuged and plasma samples were removed. Immediately thereafter (t = 0), 0.5 μCi of [^{14}C]DG in saline, and 1 mg of glucose were added to the vials of blood, which were gently shaken. At 1, 2, 3, 4, 5, 10, and 15 min, 350-μl samples of whole blood were removed, immediately centrifuged, and placed in an ice bath. Shaking of the whole blood was continued between sampling.

From the centrifuged blood, three 10-μl aliquots were removed for the measurement of radioactivity by scintillation spectroscopy (see above), and three 20-μl aliquots were taken for the enzymatic determination of glucose (Barthelmai and Czok, 1962), using a kit provided by Sigma Chemical (St. Louis, MO, U.S.A.; Technical Bulletin No. 15-UV, revised March 1983). A plasma aliquot, or an aliquot of one of several glucose standards or of distilled water was added to 3 ml of reagent in a cuvette. Absorbance was read on a spectrophotometer at a wavelength of 340 nm, and glucose concentration was calculated from the standard curve.

Radiochemical purity of [^{14}C]DG

[^{14}C]DG was subjected to one-dimensional TLC on cellulose plates (Uniplate-MN 300 cellulose, Analtech, Newark, DE, U.S.A.) in a system containing isobutyric acid/15 M NH_4OH/glass-distilled water (66:1:33, by vol). The radiochemical produced one peak with an R_f of 0.6 (Lewis and Smith, 1969).

Statistical analysis

Means of different groups were compared statistically by analysis of variance followed by a Bonferroni t test or a Dunnett's test for multiple comparisons (Dunnett, 1964; Miller, 1966). Linear regression analysis also was applied. Data were considered statistically significant when $p < 0.05$.

RESULTS

Glucose and [^{14}C]DG exchange between red blood cells and plasma

Figure 1 illustrates data from a typical experiment in which 0.5 μCi of [^{14}C]DG and 1 mg of glucose were added to 6 ml of rat whole blood at 37°C. Prior to the additions, the plasma glucose concentration equaled 160.4 mg · dl^{-1}. At 1 min, the plasma glucose concentration equaled 194.2 mg · dl^{-1}, or 33.8 mg · dl^{-1} above the initial value, and plasma radioactivity equaled 153.0 nCi · ml^{-1}.

The incremental plasma concentrations (concentration after addition minus concentration before addition) of glucose and of [^{14}C]DG, ΔC_{plasma}, and ΔC^*_{plasma}, respectively, declined logarithmically in

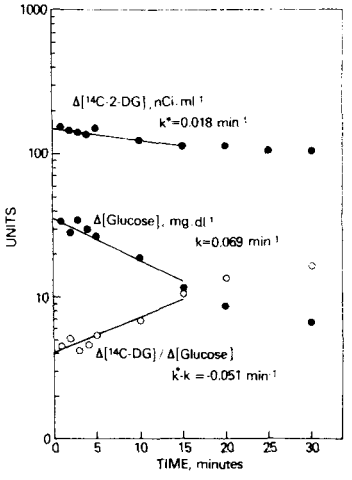

FIG. 1. Plot of incremental [^{14}C]DG and glucose plasma concentrations, ΔC^*_{plasma} and ΔC_{plasma}, respectively, in rat whole blood at 37°C against time, and of the ratio of the incremental [^{14}C]DG concentration to that of the incremental glucose concentration. Equation 2 was fit by least-squares (Knott and Shrager, 1972) to the glucose and [^{14}C]DG concentrations between 1 and 15 min, to provide the lines in the figure, with rate constants k^* equal to 0.018 min^{-1} for [^{14}C]DG and k equal to 0.0694 min^{-1} for glucose. The line related to the ratios has a rate constant, $k^* - k = -0.051$ min^{-1}.

the 15 min after the additions to whole blood, and somewhat slower thereafter (Fig. 1). An initial logarithmic decline is to be expected for influx into red cells from plasma, according to the following equations (Heath and Rose, 1969) where k is a rate constant (min^{-1}) and t is in min,

$$d\Delta C_{plasma}/dt = k\Delta C_{plasma} \quad (2a)$$

$$\Delta C_{plasma} = \Delta C_{plasma\,(t\,=\,0)} \exp(-k \cdot t) + \text{constant} \quad (2b)$$

With time, a reduced rate of decline in the incremental plasma concentration is to be expected, due to back diffusion from red cells to plasma.

Equation 2b was fit by a least-squares method (Knott and Shrager, 1972) to the glucose and [^{14}C]DG concentrations shown in Fig. 1, in the 15 min after addition of both substances to blood, to provide the regression lines illustrated in the figure. These lines correspond to $k^* = 0.018$ min^{-1} for [^{14}C]DG and to $k = 0.0694$ min^{-1} for glucose; both rate constants differ from zero ($p < 0.05$). The ratio of the incremental concentration of [^{14}C]DG to that of glucose also is shown in Fig. 1, and can be calculated from Eq. 2a as

$$\text{Ratio} = [\Delta C^*_{plasma\,(t\,=\,0)}/\Delta C_{plasma\,(t\,=\,0)}] \exp[-(k^* - k)t] \quad (3)$$

According to Eq. 3, this ratio should increase exponentially with time. The line related to values for the ratio in Fig. 1 was derived when inserting k^* and k for [^{14}C]DG and glucose, respectively, into Eq. 3, and has a slope corresponding to $k^* - k =$

-0.0514 min^{-1}. Insertion of -0.0514 min^{-1} into Eq. 3 indicates that the ratio increased by 5.3% in the first minute following additions of [^{14}C]DG and glucose to rat whole blood at 37°C.

Table 1 summarizes results on five experiments at 37°C, and on 3 experiments at 22°C (room temperature). At 37°C, the mean k^* for [^{14}C]DG equaled 0.0133 min^{-1} and k for glucose equaled 0.0577 min^{-1}. Both means differed significantly from 0 ($p < 0.05$). The ratio of the two incremental concentrations had a mean rate constant equal to -0.0444 min^{-1}, which corresponded to an average rate of rise in the ratio by 4.4 ± 0.8% in the first minute after both substances were added to blood at 37°C. At room temperature, on the other hand, the mean k^* for [^{14}C]DG equaled 0.0044 min^{-1} ($p < 0.05$) and k for glucose equaled 0.0032 min^{-1} ($p > 0.05$). The mean rate of change of the ratio of the incremental concentrations equaled -0.13 ± 0.9% ($p > 0.05$) in the first minute and corresponded to a rate constant of 0.0012 min^{-1} ($p > 0.05$).

Animal studies

Half-lives for exchange of glucose and of [^{14}C]DG between brain and blood are <2 min in awake rats (Savaki et al., 1980; Crane et al., 1981*b*). Therefore, by about 8 min after steady-state plasma concentrations of glucose and of [^{14}C]DG are established during programmed infusion of [^{14}C]DG, equilibrium between plasma and brain will occur for both. Equilibrium within 8 min has been demonstrated for [^{14}C]DG by Sokoloff et al. (1977), and was confirmed by us in initial studies in which paired arterial and sagittal sinus plasma concentrations of [^{14}C]DG were measured during the first 14 min of [^{14}C]DG infusion. On the basis of these observations, we routinely removed four to six paired samples of arterial and sagittal sinus blood only after 14 min of infusion, at 14, 20, 26, 32, 36, or 40 min.

Steady-state plasma concentrations of glucose and of [^{14}C]DG were not established in some of the 78 experiments, despite the programmed infusion schedule for [^{14}C]DG. We therefore analyzed only those experiments (45 of the 78, 15 per age group) in which the coefficients of variation of arterial plasma glucose and [^{14}C]DG concentrations during infusion did not exceed 5% and 10%, respectively.

Table 2 presents mean physiological parameters in relation to age for the 45 animals that were included in the study. The means agree with reported values in Fischer-344 rats (Ohata et al., 1981; London et al., 1981; Takei et al., 1983). A higher plasma glucose concentration in immobilized 12-month than in 3-month-old rats has been ascribed to increased stress sensitivity of 12-month-old animals (Chiueh et al., 1980). Heart rate declined significantly with age, in agreement with previous reports and despite a rise in plasma catecholamines with age (Chiueh et al., 1980). Selection criteria for including animals in this study accounted for the constant glucose concentration during infusion. The fall in the hematocrit during the experiment probably was due to removal of blood during sampling.

Table 3 illustrates typical observations on a 12-month-old rat that was infused intravenously for 40 min with [^{14}C]DG. C_a and C_a^* are the arterial plasma concentrations of glucose and of [^{14}C]DG, respectively, and C_v and C_v^* are the respective venous plasma concentrations from sagittal sinus blood. The table demonstrates constancy of arterial plasma concentrations of glucose and of [^{14}C]DG between 14 and 40 min of infusion of tracer, as well as a small SD of the ratio, $[(C_a^* - C_v^*)/C_a^*]/[C_a - C_v)/C_a]$, in rats selected according to the criteria described above. In the 15 rats 3 months of age, the mean ± SEM for $(C_a - C_v)$ equaled 22.0 ± 0.5 mg · dl^{-1}. Taking hematocrit as 0.5 (Table 2) and average cerebral blood flow as 1.0 ml · g^{-1} · min^{-1} (Takei et al., 1983) gives an estimate of 11 mg · 100 g^{-1} · min^{-1} for overall brain glucose utilization, comparable to a published value of 11.6 mg · 100 g^{-1} · min^{-1} (Hawkins et al., 1974; Siesjö, 1978).

TABLE 1. *Rates of change of incremental plasma [^{14}C]DG and glucose concentrations following addition of [^{14}C]DG and glucose to rat whole blood in vitro, at each of two temperatures*

Temperature (°C)	n	Solute	Units	ΔC at 1 min	Rate constant (min^{-1})
22	3	[^{14}C]DG	nCi · ml^{-1}	197 ± 24[a]	0.0044 ± 0.0008[a]
		Glucose	mg · dl^{-1}	39 ± 5[a]	0.0032 ± 0.0090
		DG/Glu			0.0012 ± 0.0097
37	5	[^{14}C]DG	nCi · ml^{-1}	154 ± 12[a]	0.0133 ± 0.0020[a]
		Glucose	mg · dl^{-1}	26 ± 7[a]	0.0577 ± 0.0084[a]
		DG/Glu			−0.0444 ± 0.0069[a]

Quantities added are given in text, and result in incremental concentrations at 1 min, ΔC (1 min), noted in the table. The values k^* and k are defined by Eq. 2, and equal rate constants for [^{14}C]DG and glucose, respectively. The percent change in the ratio of incremental concentrations, in the first min following the additions, is obtained by inserting k^* and k into Eq. 3.

[a] Mean ± SEM; differs significantly from zero ($p < 0.05$).

TABLE 2. *Parameters of awake Fischer-344 rats at different ages*

Parameter	Age (months)		
	3	12	24
Number of animals	15	15	15
Body weight (g)	222 ± 5	385 ± 7[a]	381 ± 6
Body temperature (°C)	36.0 ± 0.2	35.9 ± 0.1	35.7 ± 0.1
Heart rate (beats · min $^{-1}$)	427 ± 9	392 ± 8[a]	398 ± 8
Systolic blood pressure (mm Hg)	140 ± 2	147 ± 2[a]	136 ± 2[a]
Diastolic blood pressure (mm Hg)	94 ± 3	94 ± 2	90 ± 2
Arterial pH	7.40 ± 0.01	7.40 ± 0.01	7.41 ± 0.01
P_aCO_2 (mm Hg)	37.1 ± 0.3	36.2 ± 0.5	34.7 ± 0.4
P_aO_2 (mm Hg)	85.7 ± 0.9	85.5 ± 1.1	88.7 ± 1.4
Arterial hematocrit (fraction)			
Preinfusion	0.51 ± 0.00	0.51 ± 0.00	0.52 ± 0.00
Final/preinfusion	0.89 ± 0.01	0.91 ± 0.01	0.92 ± 0.01
Arterial plasma glucose (mg · dl $^{-1}$)			
Preinfusion	128 ± 3	145 ± 4[a]	150 ± 4
Final/preinfusion	1.03 ± 0.02	1.01 ± 0.02	1.00 ± 0.01

Data are from animals in which coefficients of variation of arterial plasma glucose and [^{14}C]DG concentrations did not exceed 5% or 10%, respectively, during 14–40 min of intravenous infusion of [^{14}C]DG. Dunnett's procedure was used for multiple comparisons (Dunnett, 1964). Results are means ± SEM.

[a] Differs significantly from mean at previous age ($p < 0.05$).

This correspondence indicates that the values for $C_a - C_v$ as determined in this study are not unreasonable.

Table 4 presents mean and SEM values for the ratio, $[(C_a^* - C_v^*)/C_a^*]/[(C_a - C_v)/C_a]$, in 15 rats each, at 3, 12, and 24 months of age. The mean ratio equaled 0.502 ± 0.015 (SEM) at 3 months, and was reduced significantly by 9.0% at 12 months ($p < 0.05$) and by 16.6% at 24 months ($p < 0.01$).

The coefficient of variation (CV) of the mean ratio (SD/mean) ranged from 5.5% at 24 months to 11.6% at 3 months. However, the CV of the lumped constant which is used to calculate $rCMR_{glc}$, and which is equivalent to the ratio (see Discussion below), is reported to equal 21.3% in adult awake rats (Sokoloff et al., 1977). Because the smaller CV values in our study (Table 4) might be due to our selecting only experiments in which steady-state conditions were established such that the CV values of arterial plasma glucose and of [^{14}C]DG concentrations during infusion did not exceed 5% and 10%, respectively (see Results), we recalculated mean values for the ratios in Table 4 without these conditions for all of the 78 experiments. In this case, the means and CV values for the ratios were as follows: at 3 months, n = 38 and ratio = 0.529 ± 0.025 (SEM) (CV = 26.7%); at 12 months, n = 18 and ratio = 0.467 ± 0.008 (CV = 7.2%); at 24 months, n = 18 and ratio = 0.423 ± 0.010 (CV = 10.0%). Thus, loosening the restrictions for a steady state during infusion increased the CV of the mean ratio by a factor of 2 at 3 months of age, to a value somewhat larger than that reported by Sokoloff et al. (1977), and to a lesser extent at the other ages. For the 78 experiments, the mean ratio at 24 months was significantly less ($p < 0.01$) than the means at 3 and at 12 months, but the mean at 12 months did not differ significantly ($p > 0.05$) from the mean at 3 months of age.

DISCUSSION

The lumped constant, which is required to calculate $rCMR_{glc}$ from steady-state rates of incorporation of [^{14}C]DG and of glucose from cerebral arterial blood into brain, is defined by Sokoloff et al. (1977). It is given as follows for steady-state plasma and brain concentrations of glucose and [^{14}C]DG, where C_A and C_A^* are arterial whole blood concentrations of glucose and of [^{14}C]DG, respectively; C_V and C_V^* are the respective venous whole blood concentrations obtained from the superior sagittal

TABLE 3. *Plasma concentrations of [^{14}C]DG and of glucose, during intravenous infusion of [^{14}C]DG in a 12-month-old rat*

Infusion time (min)	Plasma [^{14}C]DG			Plasma glucose			Extraction ratio
	C_a^*	C_v^* (nCi · ml $^{-1}$)	$(C_a^* - C_v^*)/C_a^*$	C_a	C_v (mg · dl $^{-1}$)	$(C_a - C_v)/C_a$	$[(C_a^* - C_v^*)/C_a^*]/[(C_a - C_v)/C_a]$ (nCi · dl $^{-1}$ · mg $^{-1}$)
0				143			
14	93.2	85.4	0.084	131	109	0.167	0.498
20	90.8	84.0	0.075	130	109	0.162	0.464
26	86.9	78.4	0.097	134	110	0.180	0.540
32	80.7	72.4	0.103	138	109	0.207	0.497
36	83.0	74.7	0.100	143	113	0.213	0.469
40	81.2	77.0	0.051	140	123	0.122	0.419
Mean:	86.0	78.7	0.085	136	112	0.175	0.481
SD:	5.2	5.1	0.020	5	5	0.003	0.041

C^* and C are concentrations of [^{14}C]DG and of glucose, respectively. Arterial plasma is denoted by subscript a, sagittal sinus venous plasma by subscript v.

TABLE 4. *Ratio of arteriovenous plasma concentration differences, divided by arterial concentrations, for [^{14}C]DG and glucose at different ages in Fischer-344 rats*

Age (months)	Number of animals	$[(C_a^* - C_v^*)/C_a^*]/[(C_a - C_v)/C_a]$ Mean ± SEM
3	15	0.502 ± 0.015
12	15	0.456 ± 0.007[a]
24	15	0.418 ± 0.006[b,c]

Arterial and sagittal sinus venous plasma concentrations are noted by subscripts a and v, respectively. The * denotes tracer. Bonferroni *t* statistics were employed for statistical analysis (Miller, 1966).

[a]Differs from mean at 3 months: $^{a}p < 0.05$; $^{b}p < 0.01$; [c]differs from mean at 12 months: $p < 0.01$.

sinus; and the other terms are defined above for arterial and venous plasma concentrations,

$$\text{Lumped constant} = \frac{C_A^*/C_A}{C_a^*/C_a} \frac{(C_A^* - C_V^*)/C_A^*}{(C_A - C_V)/C_A} \quad (4)$$

In the present study, plasma but not whole blood concentrations of glucose and of [^{14}C]DG were measured in arterial and sagittal sinus venous samples. On removal from the rat, the samples were placed in precooled vials and centrifugation was initiated within 10 s. We show below that it is possible to derive the lumped constant from plasma concentrations as measured under the conditions of this experiment.

Consider arterial blood as it passes through brain capillaries and is drained into the superior sagittal sinus. Let fractional hematocrit = h. [^{14}C]DG and glucose are removed during the passage, changing their respective plasma concentrations from C_a^* to C_v^* and from C_a to C_v, and their respective whole blood concentrations from C_A^* to C_V^* and from C_A to C_V. If no exchange between plasma and red blood cells takes place in venous blood, then, by definition,

$$(C_a^* - C_v^*)(1 - h) = C_A^* - C_V^* \quad (5a)$$

$$(C_a - C_v)(1 - h) = C_A - C_V \quad (5b)$$

Dividing Eq. 5a by Eq. 5b gives

$$(C_a^* - C_v^*)/(C_a - C_v) = (C_A^* - C_V^*)/(C_A - C_V) \quad (6)$$

The in vitro experiments summarized in Table 1 indicate that, when [^{14}C]DG and glucose are added to rather than are removed from rat whole blood, the ratio of incremental concentrations does not change significantly with time at room temperature, but increases by an average of 4.4% in the first minute at 37°C. The rate constant k (see Eq. 2) for the change in the incremental plasma concentration of glucose at 37°C, equal to 0.058 min^{-1}, is comparable to the in vivo rate of transfer of specific glucose activity from plasma to rat erythrocytes, 0.048 min^{-1} (Heath and Rose, 1969). Monosaccharides, including glucose, are transported by facilitated diffusion at rat erythrocytes, although more slowly than at human erythrocytes (Clausen et al., 1973; Chiba et al., 1974; Wagner et al., 1984). Our study shows that the rate of [^{14}C]DG transfer is less than that of glucose at 37°C, and confirms that glucose transport is temperature-dependent (Sen and Widdas, 1962; Wagner et al., 1984).

After [^{14}C]DG and glucose are removed from rat whole blood as it passes through the brains, the rate of change of the ratio of decremental concentrations should be at least as slow as the rate of change of incremental concentrations noted in the in vitro experiments at room temperature (Table 1), as blood was collected in precooled vials and centrifugation was commenced within 10 s of collection. Thus, to within 1%, Eqs. 5 and 6 are valid for the experimental conditions. Substituting Eq. 6 into Eq. 4 gives

$$\text{Lumped constant} = [(C_a^* - C_v^*)/C_a^*]/[(C_a - C_v)/C_a] \quad (7)$$

Consequently, the lumped constant is identical to the ratios that are given in Table 4 at each of three ages. The mean ratio equals 0.502 ± 0.015 in 3-month-old awake Fischer-344 rats, and declines by 16.6% between 3 and 24 months, to 0.418 ± 0.006. The value at 3 months compares with reported values for the lumped constant of 0.464 ± 0.025 in awake Sprague-Dawley rats and of 0.497 ± 0.075 in awake Wistar rats (Sokoloff et al., 1977; Sokoloff, 1979; Crane et al., 1981*a*), further supporting the equivalence of the ratios in Table 4 to the operational lumped constant.

In view of Eq. 7, it is likely that the lumped constant declines by about 16.6% between 3 and 24 months of age in the awake rat. Consequently, most reported age declines in $rCMR_{glc}$, by about 20% or less (Smith et al., 1980; London et al., 1981), probably are artifactual, as they were calculated with an age-invariant lumped constant. Whereas 12 of 17 gray matter regions in the Fischer-344 rat demonstrated statistically significant age declines in $rCMR_{glc}$ between 3 and 24 months ($p < 0.05$) (London et al., 1981), with the age-related values for the lumped constant estimated from Table 4, only one region, the inferior colliculus, retains a statistically significant age difference at $p < 0.05$ (Rapoport, 1983).

Ohata et al. (1981) found no significant age declines in rCBF between 3 and 12 months in awake Fischer-344 rats, consistent with our conclusion that $rCMR_{glc}$ does not decline in this period. Constancy of coupling between rCBF and $rCMR_{glc}$ during aging also is consistent with observations that directly measured whole brain CBF, $CMRO_2$, and CMR_{glc} in awake Fischer-344 rats, as well as the ratios of these parameters, do not differ significantly between 3 and 24 months of age (Takei et al., 1983).

As far as we know, the lumped constant has not been examined previously in relation to aging. It is reported to be unaffected by anesthesia or by hypercapnia, but to fall in extreme hyperglycemia and to rise in acute hypoglycemia and during ischemia (Ginsberg and Reivich, 1979; Sokoloff, 1979; Schuier et al., 1981; Suda et al., 1981; Pardridge et al., 1982). The arterial plasma glucose concentrations in the present study (Table 2) were far from the hyperglycemic or hypoglycemic levels that have been reported to alter the lumped constant. However, theoretical considerations indicate that the lumped constant might decrease slightly even with small increments in plasma glucose (Crane et al., 1983), and plasma glucose was elevated significantly in 12- and 24-month-old rats as compared to 3-month-old animals (Table 2).

An age change in the lumped constant may also reflect alterations in one or several parameters that compose that term, e.g., the Michaelis-Menten constants and maximal velocities of phosphorylation of [^{14}C]DG and of glucose by brain hexokinase; the fraction of glucose that, once phosphorylated by brain, is metabolized further and not dephosphorylated by glucose-6-phosphate; distribution volumes of [^{14}C]DG or of glucose between blood and brain (Sokoloff et al., 1977; Crane et al., 1983). Altered distribution volumes might be caused by changes in high- and low-affinity transport systems for glucose and for [^{14}C]DG at the blood–brain barrier (Gjedde, 1981), or by age differences in brain composition or glucose content (Medvedeva, 1937; ZS-Nagy et al., 1981; Crane et al., 1983).

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REFERENCES

Barthelmai W. and Czok R. (1962) Enzymatische Bestimmungen der Glucose in Blut, Liquor und Harn. *Klin. Wochenschr.* **40**, 585–589.

Chiba T. (1974) Effect of sulfur-containing compounds on experimental diabetes. XI. Effect of thiol and disulfide compounds on glucose transfer into red blood cells. *Chem. Pharmacol. Bull.* **22**, 439–445.

Chiueh C. C., Nespor S. M., and Rapoport S. I. (1980) Cardiovascular, sympathetic and adrenal cortical responsiveness of aged Fischer-344 rats to stress. *Neurobiol. Aging* **1**, 157–163.

Clausen T., Harving H., and Dahl-Hansen A. B. (1973) The relationship between the transport of glucose and cations across cell membranes in isolated tissues. VIII. The effect of membrane stabilizers on the transport of K^+, Na^+ and glucose in muscle, adipocytes and erythrocytes. *Biochim. Biophys. Acta* **298**, 393–411.

Coleman G. L., Barthold S. W., Osbaldiston G. W., Foster S. J., and Jonas A. M. (1977) Pathological changes during aging in barrier-reared Fischer-344 male rats. *J. Gerontol.* **32**, 258–278.

Crane P. D., Pardridge W. M., Braun L. D., Nyerges A. M., and Oldendorf W. H. (1981*a*) The interaction of transport and metabolism on brain glucose utilization: a reevaluation of the lumped constant. *J. Neurochem.* **36**, 1601–1604.

Crane P. D., Pardridge W. M., Braun L. D., and Oldendorf W. H. (1981*b*) A comparison of the blood-brain barrier transport and hexokinase kinetics of 2-fluoro-2-deoxy-D-glucose and 2-deoxy-D-glucose. *Abstr. Soc. Neurosci.* **7**, 242.

Dunnett C. W. (1964) New tables for multiple comparisons with a control. *Biometrics*, **20**, 482–491.

Ginsberg M. D. and Reivich M. (1979) Use of the 2-deoxyglucose method of local cerebral glucose utilization in the abnormal brain: evaluation of the lumped constant during ischemia. *Acta Neurol. Scand.* **60** (Suppl. 72), 226–227.

Gjedde A. (1981) High- and low-affinity transport of D-glucose from blood to brain. *J. Neurochem.* **36**, 1463–1471.

Hawkins R. A., Miller A. L., Cremer J. E., and Veech R. L. (1974) Measurement of the rate of glucose utilization by rat brain in vivo. *J. Neurochem.* **23**, 917–923.

Heath D. F. and Rose J. G. (1969) The distribution of glucose and ^{14}C glucose between erythrocytes and plasma in the rat. *Biochem. J.* **112**, 373–377.

Jones M. D. Jr., Rosenberg A. A., Simmons M. A., Molteni R. A., Koehler R. C., and Traystman R. J. (1982) Oxygen delivery to the brain before and after birth. *Science* **216**, 324–325.

Knott G. D. and Schrager R. I. (1972) On-line modeling by curve fitting, in *Computer Graphics: Proc. SIGGRAPH Computers in Medicine Symposium, ACM, SIGGRAPH Notices* **6**, 138–151.

Lewis B. A. and Smith F. (1969) Sugars and derivatives, in *Thin Layer Chromatography* (Stahl E., ed), pp. 807–837. Springer-Verlag, Berlin.

London E. D., Nespor S. M., Ohata M., and Rapoport S. I. (1981) Local cerebral glucose utilization during development and aging of the Fischer-344 rat. *J. Neurochem.* **37**, 217–221.

Medvedeva, N. (1937) Les changements vitaux de la composition protéique des tissus et l'action rajeunissante du serum hemolytique. *Med. J. Acad. Sci. USSR* **7**, 793–800.

Miller R. G. (1966) *Simultaneous Statistical Inference*, pp. 57–70. McGraw-Hill, New York.

Ohata M., Sundaram U., Fredericks W. R., London E. D., and Rapoport S. I. (1981) Regional cerebral blood flow during development and ageing of the rat brain. *Brain* **104**, 319–332.

Pardridge W. M., Crane P. D., Mietus L. J., and Oldendorf W. H. (1982) Kinetics of regional blood-brain barrier transport and brain phosphorylation of glucose and 2-deoxyglucose in the barbiturate-anesthetized rat. *J. Neurochem.* **38**, 560–568.

Patlak C. S. and Pettigrew K. D. (1976) A method to obtain infusion schedules for prescribed blood concentration time courses. *J. Appl. Physiol.* **40**, 458–463.

Raichle M. E., Grubb R. L. Jr., Gado M. H., Eichling J. O., and Ter-Pogossian M. M. (1976) Correlation between regional cerebral blood flow and oxidative metabolism. In vivo studies in man. *Arch. Neurol.* **33**, 523–526.

Rapoport S. I. (1983) Summary and discussion, in *Biological Aspects of Alzheimer's Disease* (Katzman R., ed), pp. 329–334. Banbury Report No. 15, Cold Spring Harbor Laboratory, Cold Spring Harbor.

Reivich M. (1974) Blood flow metabolism couple in brain. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **53**, 125–140.

Savaki H. E., Davidsen L., Smith C., and Sokoloff L. (1980) Measurement of free glucose turnover in brain. *J. Neurochem.* **35**, 495–502.

Schuier F. S., Orzi F., Suda S., Kennedy C., and Sokoloff L. (1981) The lumped constant for the ^{14}C-deoxyglucose method in hyperglycemic rats. *J. Cereb. Blood Flow Metab.* **1** (Suppl. 1), S63.

Sen A. K. and Widdas W. F. (1962) Determination of the temperature and pH dependence of glucose transfer across the human erythrocyte membrane measured by glucose exit. *J. Physiol. (Lond.)* **160**, 392–403.

Siesjö B. K. (1978) *Brain Energy Metabolism*, pp. 1–607. John Wiley, Chichester.

Smith C. B., Goochee C., Rapoport S. I., and Sokoloff L. (1980) Effects of ageing on local rates of cerebral glucose utilization in the rat. *Brain* **103**, 351–365.

Sokoloff L. (1978) Local cerebral energy metabolism: its relationships to local functional activity and blood flow, in *Cerebral Vascular Smooth Muscle and Its Control. Ciba Foundation Symposium* **56**, 171–197.

Sokoloff L. (1979) The ^{14}C-deoxyglucose method: four years later. *Acta Neurol. Scand.* **60** (Suppl. 72), 640–649.

Sokoloff L., Reivich M., Kennedy C., Des Rosiers M. H., Patlak C. S., Pettigrew K. D., Sakurada O., and Shinohara M. (1977) The 14C-deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* **28**, 897–916.

Suda S., Shinohara M., Miyaoka M., Kennedy C., and Sokoloff, L. (1981) Local cerebral glucose utilization in hypoglycemia. *J. Cereb. Blood Flow Metab.* **1** (Suppl. 1), S–62.

Takei H., Fredericks W. R., London E. D., and Rapoport S. I. (1983) Cerebral blood flow and oxidative metabolism in conscious Fischer-344 rats of different ages. *J. Neurochem.* **40**, 801–805.

Wagner R., Zimmer G., and Lacko L. (1984) An interspecies approach to the investigation of the red cell membrane glucose transporter. *Biochem. Biophys. Acta* **771**, 99–102.

Zeman W. and Innes J. R. M. (1963) *Craigie's Neuroanatomy of the Rat*, pp. 1–230. Academic Press, New York.

ZS-Nagy I., Nagy K., ZS-Nagy V., Kalmar A., and Nagy E. (1981) Alterations in total content and solubility characteristics of proteins in rat brain and liver during ageing and centrophenoxine treatment. *Exp. Gerontol.* **16**, 229–240.