Technetium-99m-Pertechnetate as a Whole Blood Marker for Brain Perfusion Studies

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In the brain, diffusible ^{99m}Tc-pertechnetate behaves as an intravascular indicator because it is confined within the circulation by the blood-brain barrier, allowing its use for noninvasive dynamic evaluation of cerebral circulation. For this application ^{99m}Tc has often been claimed to be a plasma marker. This study examines the validity of such a claim which has not yet been proven in vivo. **Methods:** The relative amount of ^{99m}Tc in the red cells circulating in large vessels was compared to the corresponding hematocrit (LV Hct) during the rapid (t/2 = 1.98 min) and slow (t/2 = 84 min) phases of ^{99m}Tc disappearance from the circulation after bolus intravenous injection. These comparisons were performed on rats at 2 (n = 3), 5 (n = 6), 10 (n = 6) and 20 (n = 9) sec after intravenous injection for the rapid phase and 5 (n = 5), 30 (n = 4), 60 (n = 6) and 120 (n = 6) min after intravenous injection for the slow phase. **Results:** The results show that the relative amount of intravascular ^{99m}Tc fixed to red cells did not differ statistically from LV Hct until at least 1 hr after intravenous administration. This homogeneous distribution of ^{99m}Tc in blood was indisputable during the first 20 sec but became progressively less evident and disappeared after 2 hr. Such behavior was attributed to a progressive increase of free ^{99m}Tc, which, in whole blood, amounted to 4% at 20 sec and 25% at 2 hr after injection. **Conclusion:** Because it is a 96% whole blood marker early after intravenous administration, ^{99m}Tc is a reliable agent for first-pass studies of whole blood circulation in the brain.

Key Words: technetium-99m-pertechnetate; blood marker; brain perfusion

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For many years, ^{99m}Tc-pertechnetate (^{99m}Tc) distribution in the brain was known to be limited to the vascular compartment (*1,2*). Accordingly, ^{99m}Tc has been considered to behave in the brain like an intravascular indicator per se which does not need to be bound to albumin or red blood cells before its administration. This particularity has justified the use of radionuclide cerebral angiography by intravenous bolus injection of ^{99m}Tc (*3,6*). Such an angiography also provides a time-activity curve which characterizes the first-pass of the indicator through the brain vasculature, from which the calculation of the circulatory mean transit time (MTT) can be derived (*3,5–8*).

Whatever its mode of calculation, MTT depends on what substrate (plasma and/or red cells) is labeled by ^{99m}Tc in the circulating blood. Indeed, as indicated by the cerebral hematocrit which is typically lower than that of large systemic vessels (*9*), the conservation of the red blood cell mass in brain vessels is due to the shorter mean transit time of the axial red blood cells than that of the surrounding plasma (*10*). From equilibrium dialysis data at 37°C and pH 7.4, it is already known that approximately 80% of ^{99m}Tc in plasma is bound to proteins (*11*). Up to now and despite its most common use in perfusion studies, the binding ratio of ^{99m}Tc between cellular and noncellular components of the whole blood has not yet been determined in vivo.

This study was undertaken to demonstrate the relevance of ^{99m}Tc as an homogeneous radioactive marker of the whole blood. For this purpose, fractionation of activity between cellular and noncellular components of blood was investigated from 2 sec to 2 hr after intravenous injection of ^{99m}Tc.

MATERIALS AND METHODS

Normal adult female Sprague-Dawley rats (OFA, IFFA, CREDO, France) weighing 200–225 g were provided with water ad libitum but deprived of food at least 12 hr before the experiment. They were anesthetized by an intraperitoneal injection of 2,2,2 tribromoethanol (Fluka, Switzerland) at a dose of 0.025 g/100 g body weight and heparinized (1 U/g).

In a preliminary experiment, the mean whole-body blood volume was measured in a group of 10 rats using ^{51}Cr-tagged red cells and ^{125}I-labeled human serum albumin according to the procedure described by Cremer and Seville (*9*).

A first series of experiments was performed to assess the ^{99m}Tc distribution in blood after full mixing in the circulation following bolus injection of 370 kBq (10 μCi) in a femoral vein. Animals were killed by decapitation 5 (n = 5), 30 (n = 4), 60 (n = 6) and 120 min (n = 6) after injection. At each decapitation, about 5 ml of blood was collected under pulsatile conditions from the large vessels of the thoracic extremity of the body. Blood was first sampled in two micro-hematocrit tubes and processed for large-vessel hematocrit (LV Hct) readings. Thereafter, 1 ml of blood was transferred to a counting vial. The remaining blood was centrifuged and 1 ml of plasma was then transferred to another

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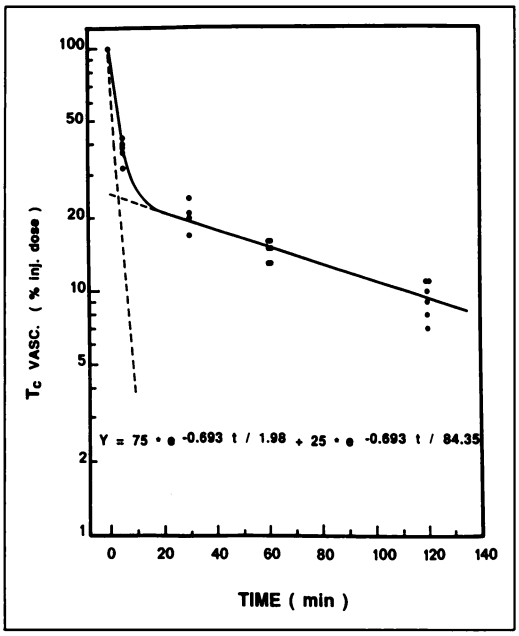

FIGURE 1. Curve of ^{99m}Tc disappearance from the circulation. This curve fits a biexponential model in which 75% of the activity (Y) disappears rapidly (t/2 = 1.98 min), while the remaining 25% disappears slowly (t/2 = 84.35 min).

counting vial. Both whole blood and plasma vials as well as an aliquot (1/100) of the injected dose were assayed for radioactivity in a gamma scintillation well counter (Canberra, Meriden, CT).

A second series of experiments was performed before full mixing of ^{99m}Tc in the circulation in order to assess the influence of mixing of ^{99m}Tc distribution in the blood. Blood from a common carotid artery was rapidly sampled in capillary tubes for microhematocrit respectively at 2 (n = 3), 5 (n = 6), 10 (n = 6) and 20 (n = 9) sec after the same ^{99m}Tc bolus injection as described above. After centrifugation and LV Hct readings, the tubes were frozen and cut at the limit between cells and plasma. Both parts were assayed for radioactivity in the same well scintillation counter as mentioned before.

RESULTS

Disappearance of Technetium-99m from the Circulation

The mean whole-body blood volume determined by ^{51}Cr-labeled red cells and ^{125}I serum albumin in rats (n = 10) with a mean body weight of 211 ± 6 (s.d.) g was found to be 14.3 ± 0.6 (s.d.) ml. From this value and the blood counting data of the first series of experiments, the fraction (%) of ^{99m}Tc remaining in the circulating blood (Y) was calculated for each experimental time (t). The resulting ^{99m}Tc disappearance curve (Fig. 1) fitted a biexponential pattern ($Y = 75 * e^{-0.3506t} + 25 * e^{-0.008215t}$) which allowed to separate a "rapid phase" (t/2 = 1.98 min) and a "slow phase" (t/2 = 84.35 min) concerning 75% and 25%, respectively, of the injected ^{99m}Tc.

Technetium-99m Blood Distribution During the Slow Phase of Disappearance

The intravascular distribution of ^{99m}Tc (^{99m}Tc vasc) between cellular (^{99m}Tc RBC) and noncellular (^{99m}Tc Pl) components of blood was evaluated as follows from the data of the first series of experiments. The red blood cell ^{99m}Tc activity was given by:

$$^{99m}\text{Tc RBC (cpm} \cdot \text{ml}^{-1}\text{ blood)} =$$

$$^{99m}\text{Tc vasc (cpm} \cdot \text{ml}^{-1}\text{ blood)} -$$

$$(1 - \text{Hct})\ ^{99m}\text{Tc Pl (cpm} \cdot \text{ml}^{-1}\text{ plasma).} \quad \text{Eq. 1}$$

In an equilibrium dialysis situation, 80% of ^{99m}Tc in plasma was found to be bound mainly to albumin while the remaining 20% was free (*11*). Assuming that such a binding ratio was also applicable in vivo, the plasma ^{99m}Tc activity (^{99m}Tc Pl) was given by bound ^{99m}Tc Pl (^{99m}Tc Pl_b) + free ^{99m}Tc Pl (^{99m}Tc Pl_f) with:

$$^{99m}\text{Tc } Pl_b \text{ (cpm} \cdot \text{ml}^{-1}\text{ blood)} =$$

$$0.8\ (1 - \text{Hct})\ ^{99m}\text{Tc Pl (cpm} \cdot \text{ml}^{-1}\text{ plasma)} \quad \text{Eq. 2}$$

and

$$^{99m}\text{Tc } Pl_f \text{ (cpm} \cdot \text{ml}^{-1}\text{ blood)} =$$

$$0.2\ (1 - \text{Hct})\ ^{99m}\text{Tc Pl (cpm} \cdot \text{ml}^{-1}\text{ plasma).} \quad \text{Eq. 3}$$

By grouping Equations 1, 2 and 3, the following parameters were evaluated:

$$^{99m}\text{Tc intravascular } (^{99m}\text{Tc vasc}) = (1) + (2) + (3)$$

$$^{99m}\text{Tc RBC } (\%\ ^{99m}\text{Tc vasc}) = [100 \cdot (1)]/[(1) + (2) + (3)] \quad \text{Eq. 4}$$

$$^{99m}\text{Tc } Pl_b\ (\%\ ^{99m}\text{Tc vasc}) = [100 \cdot (2)]/[(1) + (2) + (3)] \quad \text{Eq. 5}$$

$$^{99m}\text{Tc } Pl_f\ (\%\ ^{99m}\text{Tc vasc}) = [100 \cdot (3)]/[(1) + (2) + (3)]. \quad \text{Eq. 6}$$

The intravascular distribution of ^{99m}Tc between RBC (Equation 4), Pl_b (Equation 5) and Pl_f (Equation 6) as a function of time is illustrated in Figure 2. The regression lines were calculated for ^{99m}Tc RBC (Y = 37.15 − 0.012X) for ^{99m}Tc Pl_b (Y = 50.26 + 0.010X) and for ^{99m}Tc Pl_f (Y = 12.6 + 0.002X). The analysis of variance underlined the almost significant probabilities for the regression coefficients to be different from zero since p values were found to be 0.07, 0.07 and 0.11, respectively. These probabilities were consistent with a slight divergence between the time evolution of ^{99m}Tc RBC and ^{99m}Tc Pl: ^{99m}Tc Pl_b and ^{99m}Tc Pl_f increased similarly at a rate of 1%/hr to the detriment of ^{99m}Tc RBC which decreased at a rate of 2%/hr. According to these results, up to 60 min about 37% of intravascular

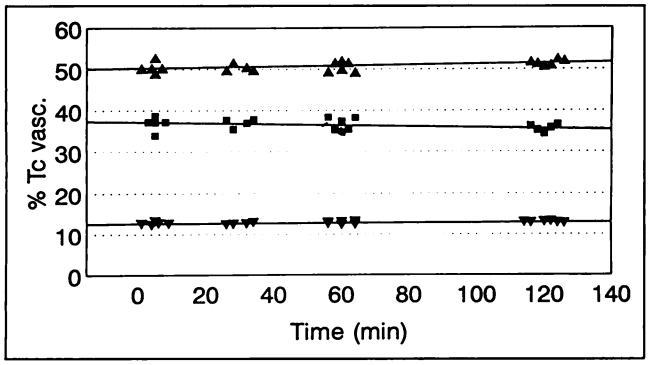

FIGURE 2. Distribution of ^{99m}Tc activity in blood. Activities in red blood cells (^{99m}Tc-RBCs) (■), bound to plasma proteins (^{99m}Tc Pl_b) (▲) and as free ^{99m}Tc in plasma (^{99m}Tc Pl_f) (▼) are expressed as percentage of intravascular activity. The fractionation of ^{99m}Tc in blood is not time constant. Technetium-99m RBCs slowly decrease in favor of ^{99m}Tc Pl_b and ^{99m}Tc Pl_f from 5 to 120 min after intravenous injection. However, these changes are not significant ($p > 0.05$).

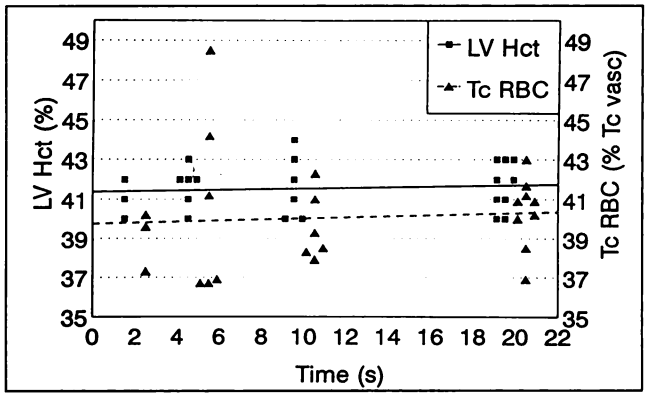

FIGURE 3. Comparison of large-vessel hematocrit values (LV Hct) (■) and red blood cell activity (^{99m}Tc RBC) (▲) expressed as percentage of intravascular activity (^{99m}Tc vasc) from 2 to 20 sec after intravenous injection. Both regression lines are parallel and underline a constant (although not significant) difference between ^{99m}Tc-RBC (interrupted line) and LV Hct (continuous line) (Table 2).

activity was located in RBC (Table 1, column 2) which represents about 42% of blood volume (Table 1, column 1). At each time, the differences observed in both these values were highly significant ($p < 0.001$).

By omitting free ^{99m}Tc activity in plasma, the percentage of red blood cell activity in labeling blood (^{99m}Tc lab · bl) was calculated as follows:

$$^{99m}\text{Tc RBC (\% } ^{99m}\text{Tc lab} \cdot \text{bl)} = [100 \cdot (1)]/[(1) + (2)]. \quad \text{Eq. 7}$$

As shown in Table 1, ^{99m}Tc RBC values resulting from Equation 7 were not found significantly different from the corresponding LV Hct values except at 120 min. Indeed, the probability of similarity was found very high ($p = 0.97$) at 5 min, lower ($p = 0.64$ and $p = 0.54$) at 30 and 60 min, respectively, and less than 0.05 at 120 min. These findings indicate that ^{99m}Tc RBC diverged progressively from LV Hct and became significantly different only at 120 min. Accordingly, bound ^{99m}Tc in blood is homogeneously distributed between RBC and plasma proteins at least up to 1 hr after tracer administration.

Technetium-99m Blood Distribution During the Rapid Phase of Disappearance

Between 2 to 20 sec after ^{99m}Tc bolus intravenous injection, the intravascular distribution of ^{99m}Tc between RBC and plasma was calculated as follows from the data on ^{99m}Tc RBC activity and ^{99m}Tc Pl activity collected in the second series of experiments:

$$^{99m}\text{Tc vasc (cpm)} = ^{99m}\text{Tc RBC (cpm)} + ^{99m}\text{Tc Pl (cpm)}, \quad \text{Eq. 8}$$

$$^{99m}\text{Tc RBC (\% } ^{99m}\text{Tc vasc)} = 100 \cdot ^{99m}\text{Tc RBC (cpm)}/^{99m}\text{Tc vasc (cpm)}. \quad \text{Eq. 9}$$

Technetium-99m RBC values as given by Equation 9 and the corresponding LV Hct values are compared in Figure 3. The regression lines were calculated for LV Hct ($Y = 41.40 + 0.16X$) and for ^{99m}Tc RBC ($Y = 39.77 + 0.27X$). Both regression coefficients were not found different from zero as tested by analysis of variance ($p = 0.66$ and 0.74 respectively). Both lines were almost parallel and showed ^{99m}Tc RBC to be constantly lower (1.63%) than LV Hct.

TABLE 1
Comparison Between Large-Vessel Hematocrits (LV Hct) and Percentages of Red Blood Cell Activities (^{99m}Tc RBC) During the Slow Phase of ^{99m}Tc Disappearance From the Circulation

n	Time (min)	LV Hct (%) (1)	^{99m}Tc RBC (% ^{99m}Tc vasc) (2)	^{99m}Tc RBC (% ^{99m}Tc lab-bl) (3)	Paired t-test (1)/(2)	Paired t-test (1)/(3)
5	5	42.2 ± 0.8	36.8 ± 1.7	42.2 ± 1.8	$p < 0.001$ (S)	$p = 0.97$ (ns)
4	30	42.0 ± 1.2	37.0 ± 1.1	42.3 ± 1.1	$p < 0.001$ (S)	$p = 0.64$ (ns)
6	60	42.5 ± 0.8	36.6 ± 1.5	41.9 ± 1.6	$p < 0.001$ (S)	$p = 0.54$ (ns)
6	120	42.3 ± 0.5	35.5 ± 0.8	41.1 ± 0.9	$p < 0.001$ (S)	$p < 0.05$ (s)

Results are mean ± s.d.; ns = not significant; s = significant.

TABLE 2
Comparison Between Large-Vessel Hematocrits (LV Hct) and Percentages of Red Blood Cell Activities (^{99m}Tc RBC) During the Rapid Phase of ^{99m}Tc Disappearance from the Circulation

n	Time (sec)	LV Hct (%)	^{99m}Tc RBC (% ^{99m}Tc vasc)	Paired t-test
3	2	41.0 ± 1.0	39.0 ± 1.5	P = 0.29 (ns)
6	5	41.7 ± 1.0	40.7 ± 4.9	P = 0.61 (ns)
6	10	41.7 ± 1.6	39.6 ± 1.7	P = 0.15 (ns)
9	20	41.7 ± 1.2	40.4 ± 1.8	P = 0.15 (ns)

Results are mean ± s.d., ns = not significant.

However, this difference was not significant at the four tested times (paired t-test) (Table 2).

DISCUSSION

When measurements were made within 2 hr after intravenous administration, the disappearance of ^{99m}Tc from the circulation was shown to fit a biphasic exponential pattern (*12*). The curve of Figure 1 illustrates this finding. It can also be described by two separate phases: a rapid phase which concerns about 75% of the injected ^{99m}Tc obviously combines the effects of its intravascular mixing and its leakage from intra- to extravascular spaces; and a slow phase which concerns the remaining 25% of ^{99m}Tc only refers to its leakage from intra- to extravascular spaces. The first and the second series of experiments are therefore adequately designed to evaluate the distribution of the remaining intravascular ^{99m}Tc between the blood components during both modes of its disappearance from the blood.

Since 20% of ^{99m}Tc in plasma can be considered to be unbound (*11*), our results for the slow phase (from 5 to 120 min after injection) show that during its intravascular life, 87.4% of ^{99m}Tc is bound to red cells and plasma proteins while 12.6% is free (Fig. 2, extrapolated values at t = 0). Technetium-99m can therefore be considered as a 87% blood marker only if binding is evenly distributed between red cells and plasma. This is demonstrated by our comparison between the percentages of activity in red cells relative to bound activity in the whole blood and the corresponding large-vessel hematocrit. Table 1 shows that LV Hct and ^{99m}Tc RBC (% ^{99m}Tc lab · bl) are statistically equivalent except at 120 min. Such a finding is consistent with a similar binding capacity for red cells and plasma proteins. This is in agreement with the work of Hays and Green (*11*) which concluded that red cell membranes and plasma proteins bind ^{99m}Tc with a similar degree of affinity and kinetic stability. Such a conclusion implies an equal diffusion of the ligand away from both red cell membranes and plasma proteins. In this context, the significant difference observed at 120 min between LV Hct and ^{99m}Tc RBC (% ^{99m}Tc lab · bl) is likely to be due to an increase of the amount of free ^{99m}Tc in plasma which at that time can be calculated at 25% (see Appendix).

During the rapid phase of ^{99m}Tc disappearance, the results obtained from 2 to 20 sec after injection were characterized by a larger variability of ^{99m}Tc RBC data compared to LV Hct data, particularly at 5 sec (Table 2 and Fig. 3). According to our previous observations in rats (*13*), at 2 sec the blood samples were collected during the first wave of the dilution curve of ^{99m}Tc in the circulation while at 5 sec they were collected at the end of the first and the beginning of the second dilution waves. At 10 sec, wave phenomena were no longer observable (*13*). From these observations, we can expect that the front of the first dilution wave is relatively homogeneous and does not greatly affect the intravascular distribution of ^{99m}Tc. By contrast, the overlappings of the two first dilution waves observed at 5 sec vary depending on the individual circulatory mean transit-times (*13*). Such a variability in the ^{99m}Tc blood mixing process could interfere with its intravascular distribution as reflected by the dispersion found in the 5-sec data in Figure 3.

After this critical time, ^{99m}Tc distribution in blood was more constant (Table 2, Fig. 3). In spite of this variability, Table 2 obviously shows that ^{99m}Tc distributed between red cells and plasma in close relationship to the corresponding LV Hct. However, in Figure 3, the ^{99m}Tc regression line was constantly 1.63% lower than that of LV Hct. Although insignificant, this small difference suggests the presence of some free ^{99m}Tc already at these early times. Indeed, a similar rapid calculation (as in the Appendix) indicates that such a small difference could reflect 6.5% of free ^{99m}Tc in plasma which corresponds to 4% of free ^{99m}Tc in whole blood; this would imply that 96% of ^{99m}Tc is then bound to whole blood. The conclusion therefore arises that ^{99m}Tc in the whole blood within the first seconds after the intravenous injection is distributed as a reliable blood marker. However, such a property declines with time since our results indicate that the fraction of free ^{99m}Tc increases progressively from seconds to hours after administration.

Our results should be of great interest to investigators who use ^{99m}Tc for first-pass studies in the brain in that this tracer also allows total blood volume measurement without the need of a speculative value for cerebral hematocrit (*14*).

APPENDIX

Free Technetium Calculation

At 120 min:

$$^{99m}\text{Tc RBC (\% } ^{99m}\text{Tc vasc)} = 35.5,$$

$$^{99m}\text{Tc Pl (\% } ^{99m}\text{Tc vasc)} = 100 - 35.5 = 64.5,$$

$$\text{LV Hct (\%)} = 42.3.$$

Assuming an even binding of blood without free ^{99m}Tc activity, we can write:

$$35.5/(35.5 + X) = 0.423,$$

$$X = 48.4 \text{ (\% } ^{99m}\text{Tc vasc)},$$

where X is the ^{99m}Tc Pl (%^{99m}Tc vasc) corresponding to the above assumption. Thus the difference between measured and assumed ^{99m}Tc Pl is given by:

$$64.5 - 48.4 = 16.1 \text{ (\%}^{99m}\text{Tc vasc)}.$$

This 16.1% excess of ^{99m}Tc plasma activity therefore represents free ^{99m}Tc in blood that amounts to 25% of ^{99m}Tc plasma activity (16.1/64.5 = 0.25).

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