special communication

Appropriate background correction for DTPA aerosol clearance

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Mason, G. R., A. M. Peters, M. J. Myers, and J. M. B. Hughes. Appropriate background correction for DTPA aerosol clearance. *J. Appl. Physiol.* 84(3): 1103–1107, 1997.—Measurement of the clearance rate of inhaled aerosols of ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA) from distal airway to pulmonary capillary is a sensitive technique for the detection of lung injury. As the solute diffuses across the blood-gas barrier, the concentration in circulating blood increases, giving rise to a background signal superimposed on the signal from residual DTPA in the airway. Background subtraction is conventionally based on the thigh, but this tissue has the disadvantage in that its composition, in terms of the relative volumes of its extracellular extravascular and intravascular compartments (a ratio of ~4:1), is quite different from that of the lung (<1:6). With comparison to the thigh, we examined alternative regions for background, liver, and cranium, which have extravascular-to-intravascular compartment ratios much closer to these for the lung, to determine the most appropriate background for correction of the pulmonary signal. From 1 min after intravenous injection of ^{99m}Tc-DTPA, the time-activity curves recorded by a gamma camera over the liver and lung in a group of otherwise normal cigarette smokers decreased up to 30 min after injection, with time courses that could essentially be superimposed on each other; the curve recorded over the thigh with a separate scintillation probe continued to increase. The curve recorded over the cranium had a time course similar to that for the liver and lung. Following aerosol inhalation, the lung clearance rates over the initial 7 min when background subtraction was used, based on the liver, cranium, and thigh were, respectively, 4.9 ± 2.9, 4.7 ± 2.6, and 5.4 ± 3.4 (SD) %/min, compared with 4.1 ± 2.2%/min without subtraction. The corresponding values based on 30 min of data were 3.3 ± 1.4, 3.4 ± 1.4, 4.2 ± 2.3, and 2.8 ± 1.0%/min. When the liver was used for background, the lung clearance curves were clearly multiexponential, whereas thigh correction tended to give curves that were monoexponential or even convex upward on semilogarithmic axes. With an appropriate region for background, the true shape of a lung curve can be identified, which permits the study of an intervention on the clearance while it is in progress. The intravenous DTPA, required for calibrating the background regions, can be given before inhalation of the tracer.

lung aerosol clearance; 99m-technetium-diethylenetriamine pentaacetic acid; alveolar permeability

THE CLEARANCE OF SMALL HYDROPHILIC SOLUTES from the lung, following delivery by aerosol inhalation, is an established technique for assessing the integrity of the distal lung epithelium (1–3). The agent usually used is ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA) (mol wt 492), which is thought to move from airway to pulmonary capillary blood by diffusion through gaps between the epithelial cells (4–6).

As ^{99m}Tc-DTPA enters the circulation, the increasing extra-alveolar radioactivity in the lung and chest wall necessitates subtraction of background counts from the alveolar clearance curve when studies are longer than a few minutes in duration. Since the technique was first introduced, this has usually been based on a radioactivity signal recorded over the thigh with a separate scintillation probe (7, 8, 12). To scale this background signal from the thigh, the simultaneous increments in count rate are recorded over the thigh and lung after a small intravenous dose of ^{99m}Tc-DTPA is given at the completion of the lung clearance study.

Whereas the time-activity curve recorded over the lung after intravenous ^{99m}Tc-DTPA decreases, the activity over the thigh increases for at least 10 min as a result of movement of ^{99m}Tc-DTPA from the intravascular to extravascular spaces of the thigh (10). This makes it difficult to identify the time after the intravenous dose on which to base the scaling factor for the thigh background curve, before its subtraction from the lung curve. Furthermore, because of different rates of tracer equilibration between the intravascular and extravascular spaces following inhalation and intravenous injection, respectively, the scaling factor would be expected to vary with time after inhalation.

A tissue similar in composition to the lung in terms of its proportion of intravascular and extravascular space volumes or, alternatively, a tissue in which DTPA equilibrated between intravascular and extravascular spaces at the same rate as in the lung should, therefore, offer an advantage over the thigh. The thigh has an intravascular-to-extravascular volume ratio <1:5 (10), compared with the lung in which the corresponding ratio is considerably higher (11). One such tissue is the liver, which, like the lung, has a relatively small extravascular space (relative to intravascular) and in which equilibration is rapid. Another is the brain, the extravascular space of which is not accessible to DTPA because of the impermeability of the blood-brain barrier. Furthermore, the chest wall over the liver and the scalp over the brain have similar compositions to the chest

wall over the lung, with respect to intravascular and extravascular spaces.

With these theoretical considerations in mind, the aim of this paper was to evaluate the liver and cranium as alternative sites to the thigh for background correction of lung aerosol clearance curves.

METHODS

Subjects. Seven otherwise healthy cigarette smokers were studied. Smokers were chosen because they would predictably have faster clearances. Age range was 18–55 yr. All were long-term as well as current smokers and had elevated levels of exhaled carbon monoxide immediately before testing. An eighth subject, a nonsmoker, inhaled platelet-activating factor (PAF) after aerosol inhalation to illustrate the differences in background subtraction methods (previously reported, see Ref. 11). All subjects gave informed written consent to participate in the study, which was approved by the Hammersmith Hospital and Royal Postgraduate Medical School Local Research Ethics Committee and by the Administration of Radioactive Substances Advisory Committee of the United Kingdom.

Imaging. The subject was imaged supine with a gamma camera (IGE 400 A or T, interfaced to an MDS A^2 computer) positioned anteriorly over the upper abdomen and chest. Twenty megabecquerels of ^{99m}Tc-DTPA (pentetate; Amersham International, Bucks, UK) was given intravenously, and dynamic imaging was performed for 30 min at two frames per minute. An aerosol of ^{99m}Tc-DTPA was generated into a 30-liter balloon immediately before inhalation with an acorn nebulizer (OEM, Richmond, VA); as a result of settling into the balloon, the particles inhaled are median mass diameter (1.8 μm, 0.84 geometric SD). The subject inhaled the aerosol for 120 s: after breathing aerosol for 105 s, the circuit was changed to room air while the subject cleared the tubing of residual aerosol in the remaining 15 s. Imaging was continued for 30 min after inhalation was complete.

During both phases of acquisition, a scintillation probe was positioned either over the temporal region of the cranium or at midthigh. In the latter position, it was angled away from the bladder to avoid a signal from radioactivity in the urine.

Analysis. Separate regions of interest were placed over the whole left and whole right lungs and over the right lobe of the liver. The latter was separated from the right lung (as defined on the aerosol image) by at least two pixels on a 64 × 64 matrix. Time-activity curves were generated for both lungs and liver and, by using a dedicated minicomputer, from the cranium or thigh.

Background subtraction. The point in time at which the time activity curves over the cranium or thigh became almost parallel to the time axis (t_{const}) after intravenous ^{99m}Tc-DTPA injection was identified. The ratio of count rates from whole lung to background region at this time was recorded to provide the scaling factor. The background tissue count rate recorded after ^{99m}Tc-DTPA inhalation was then multiplied by this ratio and subtracted from the curve recorded over the lung after inhalation to generate either a cranium-corrected or thigh-corrected lung curve. For background correction based on the liver, the same scaling technique was performed by using the count rate recorded from the liver region of interest at time t_{const}.

Calculation of aerosol clearance rate. The lung clearance curve was examined for linearity on a semilogarithmic plot. The curve was fitted with a monoexponential function from the time of maximal activity after inhalation (t_{max}) to $t_{max+7\ min}$ and also from t_{max} to $t_{max+30\ min}$, thereby generating a clearance rate in units of percent of residual tracer per minute. This was performed on unsubtracted lung curves and on lung curves from which background had been subtracted by using the curves recorded from the liver, thigh, and cranium. The formula for corrected lung activity ($LA_{corrected}$) is

$$LA_{corrected} = LA - (SF \times LA)$$

where SF is scaling factor denoting LA/background activity at time = t_{const}.

Statistics. Parametric studies have been applied to the data. Values are expressed as means ± 1 SD.

RESULTS

After intravenous injection, the count rate over the lung decreased rapidly for the first 5 min and then more slowly (Fig. 1). The count rate recorded over the thigh, however, initially increased, briefly became constant at ~10 min, and then fell slowly. At 20 min, the fractional rate of decrease over the lung was still greater than over the thigh; in other words, equilibrium of tracer between intravascular and extravascular compartments in the thigh had not been reached, equilibrium in this context being defined as equalization of the respective rate constants of decline of activity in the two compartments. In contrast, the count rate recorded over the liver showed a very similar time course to that over the lung, particularly from 1 min after injection when the intravenous bolus had made its first pass through the pulmonary vascular bed. The time-activity curve recorded over the cranium was somewhat more variable than over the other three regions (Fig. 2).

To obtain a better visual comparison of the relationship of the three extrapulmonary regions to the lung, the ratio of lung activity to background activity (from 1 min after injection) is shown for three subjects in Fig. 3. In each subject, the lung-to-thigh ratio declines while the lung-to-liver or lung-to-head ratios are stable or rising slightly for the first 10 min or longer.

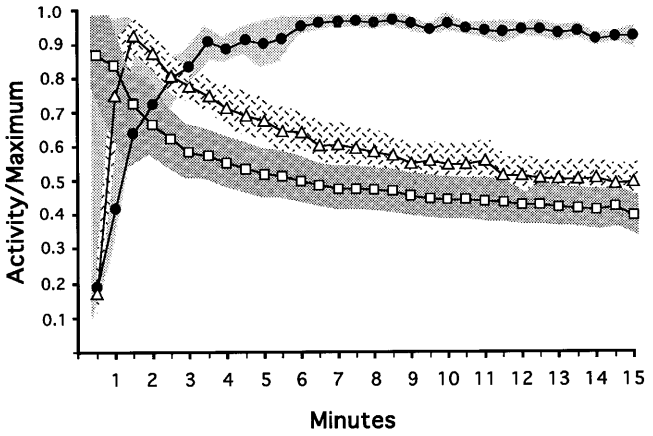

Fig. 1. Average activity (6 subjects) in lung (□), liver (△), and thigh (●) after intravenous bolus of ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA). For comparison, curves are expressed as %maximal activity in that organ. Lung activity is declining within 30 s after injection: liver has similar kinetics, offset by 60 s. Thigh activity, with a larger volume of distribution, does not begin to decline until 10 min after injection. SE indicated by shaded area.

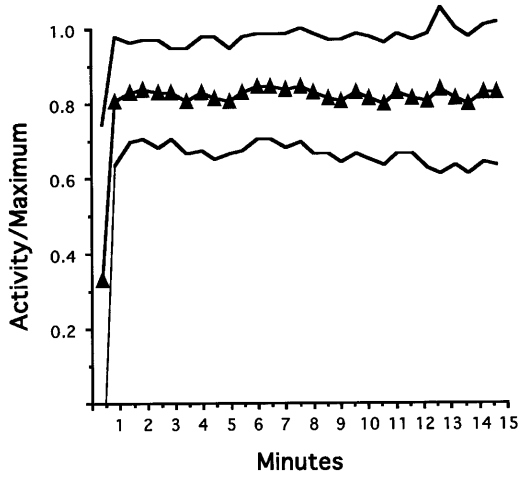

Fig. 2. Time-activity curve recorded over the cranium. Cranial activity was somewhat more variable than over the other 3 regions in Fig. 1 and is shown with SD values (solid lines).

The time course of lung activity after DTPA inhalation that is not corrected for background is shown in Fig. 4. When expressed on a logarithmic *y*-axis (i.e., semilogarithmically), the curves appear concave upward, i.e., they do not conform to a monoexponential decrease. Accordingly, fitting of a monoexponential function to the curve from t_{max} to $t_{max+7\ min}$ gave an average clearance rate of 4.1 ± 2.2 (SD) %/min and to $t_{max+30\ min}$ 2.8 ± 1.0%/min. The time course of lung activity, background corrected by using the liver, was more closely monoexponential, although, like the uncorrected curves, could still be described as multiexponential. Lung curves corrected by using the cranium were similar to liver-corrected lung curves. The clearance rates, up to $t_{max+7\ min}$, for the liver-, cranium-, and thigh-corrected lung curves were 4.9 ± 2.9, 4.7 ± 2.6, and 5.4 ± 3.4%/min, respectively, and up to $t_{max+30\ min}$ 3.3 ± 1.4, 3.4 ± 1.4, and 4.2 ± 2.3%/min, respectively (Table 1).

The effects of an intervention, PAF inhalation, on the lung clearance curves recorded before and after background correction and on the background count rates themselves are shown in Fig. 5. The intervention resulted in a sharp increase in the rate of pulmonary ^{99m}Tc-DTPA clearance. It is evident that, although the liver and thigh activity curves both increased progressively before the intervention and both showed a sharp increase immediately after the intervention, the liver curve started to fall 5 min after the intervention while the thigh curve continued to increase.

DISCUSSION

It is well known from other uses of ^{99m}Tc-DTPA in nuclear medicine (e.g., renography) that the time-activity curve recorded over the lung after intravenous injection of this tracer decreases over 20 min to about one-half the value at 1 min. This is to be expected, as the lung has an extravascular space that is relatively small compared with its intravascular space and, therefore, gives a signal that is largely intravascular. Furthermore, because of the large capillary surface area throughout the body, available for exchange of solute between intravascular and interstitial spaces, plasma tracer concentration rapidly decreases. Because inhaled ^{99m}Tc-DTPA crossing the distal lung epithelium first enters the intravascular space before it diffuses into the whole body extravascular space, the ideal background region for correction of the lung aerosol clearance curve should have a composition, in terms of relative intravascular and extravascular space volumes, identical to that of the lung or with identical regional kinetics of DTPA equilibration between the spaces. The thigh has a relatively small intravascular volume and a large extravascular volume, and the time course of DTPA equilibration is slow. After the calibrating intravenous dose of ^{99m}Tc-DTPA, therefore, the ratio of lung-to-thigh background activity decreases, i.e., the scaling factor for the thigh curve (expressed as lung-to-

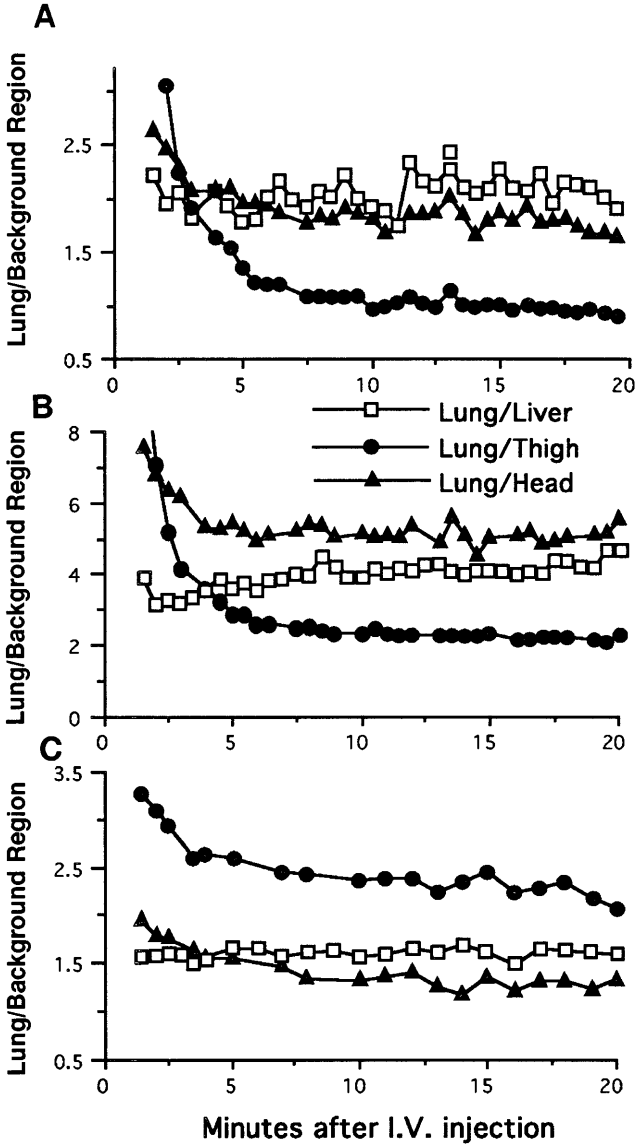

Fig. 3. Ratio of lung activity to background region activity after intravenous (iv) injection in 3 subjects. In each subject, lung-to-thigh ratio declines, whereas lung-to-liver or lung-to-head ratios are stable or rising over the 1st 10 min or longer.

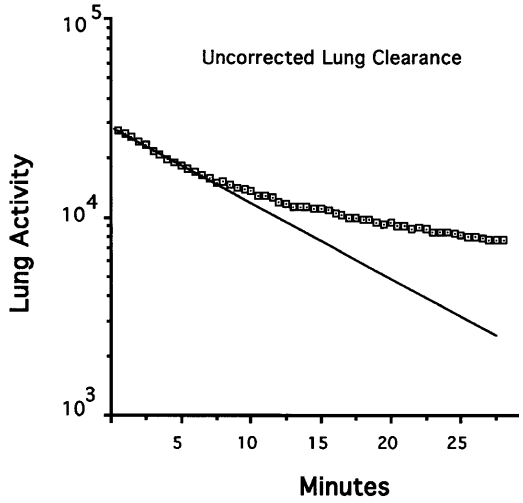

Fig. 4. Time course of background-uncorrected lung activity after DTPA inhalation in a normal smoker, expressed on a logarithmic *y*-axis. This curve appears concave upward, i.e., it does not conform to a monoexponential decline.

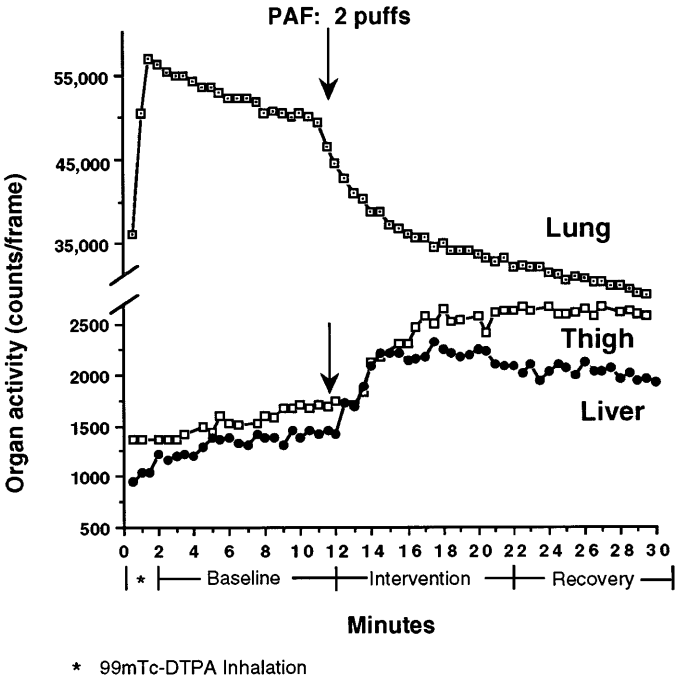

Fig. 5. ^{99m}Tc-DTPA lung clearance and appearance in liver and thigh after platelet-activating factor (PAF) administration in a nonsmoking subject. After 2-min administration of ^{99m}Tc-DTPA aerosol, baseline clearance for 10 min is 1.7%/min. During this time, there is slow accumulation in liver and thigh tissues. After PAF, there is an abrupt and transient increase in lung clearance (3.4%/min) and a rapid increase in liver activity, followed by an increase in thigh activity. Liver activity begins to decline, whereas thigh activity continues to rise. It may be appreciated that magnitude of correction is small but present in a normal subject even in the 1st 10 min. During this transient change in clearance from PAF, background subtraction from the thigh would result in an overcorrection, affecting both immediate and later portions of the study. Duration of the effect of PAF is <10 min. Clearance for the final 10 min is 1.7%/min. [From Mason et al. (11)]

thigh ratio) decreases as a function of time (Fig. 3). Equilibration of ^{99m}Tc-DTPA between intravascular and extravascular spaces in the thigh is probably not completed until ~20 min (10). The scaling factor at this time would give an accurate estimate of lung background only if, at all times after inhalation, the ratio of intravascular to extravascular activity in the thigh was the same as the ratio in the thigh at 20 min after intravenous injection, a requirement that, obviously, cannot be met. At least over the initial part of the aerosol curve such a scaling factor would overestimate background in the lung curve. Basing the scaling factor for the thigh on times after intravenous injection before equilibration has been achieved is arbitrary, and it is impossible to determine, at any given time after inhalation, what ratio of intravascular to extravascular activities is present in the thigh. On theoretical grounds, therefore, the thigh is clearly an inappropriate background region and cannot give an accurate estimation of true background over the lung arising after inhalation.

When using the scaling factor recorded at ~10 min after intravenous injection, the thigh background-corrected lung curve was convex upward on semilogarithmic display, consistent with a scaling factor, which underestimated background subtraction for the early part of the curve and/or overestimated background subtraction for the later part of the aerosol curve (probably both, since the terminal slope of the lung curve after thigh-background correction was steeper than the lung curve after liver-background correction). Table 1 demonstrates progressive slowing of DTPA clearance regardless of the method of correction. It is noteworthy that the thigh-corrected rate constant at 30 min is very similar to the uncorrected rate constant at 7 min, suggesting, intuitively but erroneously, that a correction based on the thigh may be appropriate.

Table 1. *Clearance rates for liver-, cranium-, and thigh-corrected lung curves*

	7-min Calculation, %decline/min	30-min Calculation, decline/min	Magnitude of Slowing, 30 vs. 7 min, %
Uncorrected *k*	4.1 ± 2.2	2.8 ± 1.0	32
k Liver correction	4.9 ± 2.9	3.3 ± 1.4	33
k Head correction	4.7 ± 2.6	3.4 ± 1.4	28
k Thigh correction	5.4 ± 3.4	4.2 ± 2.3	22

Values are means ± SD. *k*, clearance rate.

Because they have relatively small extravascular distribution volumes of ^{99m}Tc-DTPA compared with intravascular volumes, the liver and cranium are theoretically preferable to the thigh for background subtraction. Lung curves corrected by using the cranium or liver gave clearance values lower than the corresponding thigh background-subtracted curves, which we interpret as the result of oversubtraction when using the thigh. The problem of appropriate scaling does not arise with the liver, since the scaling factor, although not necessarily unity, is constant and not time dependent like the scaling factor for the thigh. Compared with the thigh, the scaling factor for the cranium also varied less over time after intravenous injection. Although not appearing to perform quite as well as the liver, the cranium would be a preferable alternative if a gamma camera was not available for monitoring the

lung activity, since a scintillation probe placed over the liver cannot reliably detect a liver signal free of cross talk from the lung.

Other investigators have presented evidence in support of the validity of the thigh for background subtraction. By delivering aerosol to a single lung in a pig model, Barrowcliffe et al. (1) were able to compare the thigh with the opposite lung. The count rate over the nonaerosolized lung after intravenous injection of ^{99m}Tc-DTPA showed a time course similar to that recorded over the thigh, and both gave the same background-corrected aerosol clearance curve.

Accurate background subtraction is, in general, probably not critical for routine clinical use of the technique, especially when aerosol clearance values are based on the initial 7 min of data. Nevertheless, it becomes important in clinical research, when it is desired to examine the effects of interventions on the clearance curve. An example illustrated in this paper is the effect of PAF inhalation on the lung ^{99m}Tc-DTPA clearance curve (Fig. 5) (15). In the subject illustrated here, a normal nonsmoker, it can be seen how the blood level of DTPA, as reflected by liver radioactivity, increased progressively after inhalation. After PAF inhalation, there was a transient increase in count rates from both liver and thigh regions. The count rate from the liver then fell while that from the thigh continued to rise, reflecting the difference in relative contents of extravascular space between the two tissues.

Inappropriate background subtraction distorts the shape of the lung aerosol clearance curve. In view of the interest in the significance of a biexponential clearance curve, this may be of relevance. A biexponential curve is generally regarded as indicative of severe lung damage and interpreted as the result of different rates of clearance from two separate populations of alveoli (1, 8, 9). Although there is some evidence to support this (8, 9), other explanations are at least as plausible, such as bidirectional movement of tracer across the lung epithelium (13, 14). Indeed, with appropriate background correction, we frequently see clearance curves composed of more than one exponential. Progressive oversubtraction of background, which we have shown here to result from the use of the thigh, would be expected to mask the true shape of the clearance curve.

Administration of the intravenous dose of ^{99m}Tc-DTPA, for calibration of the background signal, before ^{99m}Tc-DTPA inhalation offers some advantages. Conventionally, the intravenous dose is given after the inhalation dose, but it is more accurate to subtract a preceding signal when it is smaller than the succeeding signal from which it is subtracted. Indeed, it is not necessary to subtract the preceding signal at all, since by having a background curve with a shape identical to the background in the lung, we can use it for background subtraction at any stage of the lung curve, before or after inhalation. In other words, it can be imagined that the intravenous dose would have essentially the same effect as an early rapid transfer of ^{99m}Tc-DTPA from alveolus to blood.

In conclusion, we describe two simple but important modifications to the technique for measuring distal lung epithelial permeability by aerosol inhalation, consisting of giving the intravenous dose before inhalation and choosing a background region with an appropriate composition of extravascular and intravascular DTPA distribution volumes.

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Received 1 November 1995; accepted in final form 12 November 1997.

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