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E. G. Langenback , W. M. Foster & E. H. Bergofsky

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CALCULATING CONCENTRATION OF INHALED RADIOLABELED PARTICLES FROM EXTERNAL GAMMA COUNTING: EXTERNAL COUNTING EFFICIENCY AND ATTENUATION COEFFICIENT OF THORAX

E. G. Langenback, W. M. Foster, E. H. Bergofsky

Pulmonary/Critical Care Division, Department of Medicine, SUNY at Stony Brook, Stony Brook, New York

We determined the overall external counting efficiency of radiolabeled particles deposited in the sheep lung. This efficiency permits the noninvasive calculation of the number of particles and microcuries (μCi) from gamma-scintillation lung images of the live sheep. Additionally, we have calculated the attenuation of gamma radiation (120 keV) by the posterior chest wall and the gamma-scintillation camera collection efficiency of radiation emitted from the lung.

Four methods were employed in our experiments: (1) by light microscopic counting of discrete carbonized polystyrene particles with a count median diameter (CMD) of 2.85 μm and tagged with cobalt-57 (^{57}Co), we delineated a linear relationship between the number of particles and the emitted counts per minute (cpm) detected by well scintillation counting; (2) from this conversion relationship we determined the number of particles inhaled and deposited in the lungs by scintillation counting fragments of dissected lung at autopsy; (3) we defined a linear association between the number of particles or microcuries contained in the lung and the emitted radiation as cpm detected by a gamma scintillation camera in the live sheep prior to autopsy (external counting efficiency); and (4) we compared the emitted radiation from the lungs of the live sheep to that of whole excised lungs in order to calculate the attenuation coefficient (ac) of the chest wall. The mean external counting efficiency was 4.00×10^4 particles/cpm (5.1×10^{-3} μCi/cpm), the camera collection efficiency was 1 cpm/10^4 disintegrations per minute (dpm), and the ac had a mean of 0.178/cm. The external counting efficiency remained relatively constant over a range of particles and microcuries, permitting a more general use of this ratio to estimate number of particles or microcuries depositing after inhalation in a large mammalian lung if a similarly collimated gamma camera system is used.

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Requests for reprints should be sent to Edward H. Bergofsky, Pulmonary/Critical Care Division, Department of Medicine, SUNY at Stony Brook, Stony Brook, NY 11794.

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INTRODUCTION

Measurement of an injected or inhaled radionuclide is often used in the noninvasive evaluation of anatomic integrity, physiologic function, ventilation, perfusion, and pathologic conditions. The emitted radiation is acquired by a gamma-scintillation camera through a collimator, detected as single photons and recorded as a planar image of an organ (Lieberman, 1977). Often, serial images are acquired and stored by computer for analysis of accumulation or paucity of emitted radiation within an organ or a change of radiation detected over time indicating radionuclide shifting within or exiting from an organ (Foster et al., 1980). The determination of radionuclide concentration by measurement of emitted radiation has some inherent inaccuracies because this radiation is a function of the distribution of radionuclide in the organ, distance and counting efficiency of the camera, and attenuation of the emitted radiation by fat, muscle, bone, and other organs. Since these parameters are usually unknown, indirect methods have been employed to estimate radionuclide concentration. These methods include (1) calibrated intravenous radiation doses delivered primarily to one organ (Early and Sodee, 1985) and (2) radiolabeled aerosols delivered directly to the lungs, which are monitored for differences in the amounts of inhaled and exhaled radiolabel (Smaldone and Messina, 1985; Messina et al., 1985; Messina and Smaldone, 1985). These techniques lose accuracy due to some dissemination of the radiolabel systemically in blood, or imprecise instantaneous measurements of rapidly flowing aerosols. This is particularly problematic in quantitative studies of uptake and subsequent changes in radionuclide concentration within an organ.

We have used a direct method of determining the amount and distribution of a radionuclide in the lungs of the sheep. Through a series of noninvasive gamma-scintillation camera images and postmortem dissections, the exact amount of radionuclide in the form of polystyrene particles tagged with ^{57}Co was determined. Measurements on different sheep at various radionuclide concentrations in the lung have yielded a curve converting counts per minute acquired on the gamma-scintillation camera to actual microcuries or particles in the lung of the intact sheep. This relationship permits the calculation of the external radionuclide counting efficiency (ratios: particles/cpm and μCi/cpm) and the attenuation of the emitted gamma rays by the chest wall. It has thus been possible to standardize a number of variable parameters and quantitate radionuclide or particle dose in the lungs. This allows an accurate evaluation of uptake and change of radionuclides, for example, the radiolabeled particle deposition and clearance kinetics in the lung by serial noninvasive scintillation images over time. Anatomic and functional similarities between sheep and humans (Horsfield, 1978;

Plopper et al., 1983; McLaughlin, 1961) permit potentially relevant and valuable analogies to be made where such information cannot be directly measured in humans.

METHODS

Animals

Five Q-fever-negative, mixed-breed female sheep between 2 and 4 yrs of age and weighing from 140 to 220 lb were isolated for 1 mo prior to inhalation and their health was monitored regularly through blood tests and fecal analysis. When necessary, antibiotics and anthelmintics were administered. During the experimental period the sheep were housed indoors in separate cages and fed a diet consisting of grain, hay, alfalfa, and water. In addition, the sheep were sheared and checked for infections and parasites throughout the course of the experiments.

Drugs

The drugs administered to the animals were primarily anesthetics used during the inhalation procedure. Rompun (20 mg/ml), a xylazine with a biological time of 10 min, was administered intravenously at 0.5 mg/kg animal weight. During this period the sheep was intubated with a cuffed endotracheal tube and an intravenous solution of 0.9% sodium chloride was set up. Pentobarbital sodium solution (65 mg/ml) was administered as needed via the iv saline system. During the inhalation procedure about 20–30 ml was infused over a period of 60–90 min. The animal was maintained prone at a light level of anesthesia, which allowed it to breathe entirely on its own while tolerating the endotracheal tube. Post inhalation, the sheep was able to stand and eat within 4–5 h.

Particles

The particles used for inhalation were insoluble carbonized polystyrene spheres with greater than 80% carbon content and a count mean diameter of 3.0 ± 1.0 (SD) μm as stated by the manufacturer (3M Medical Products Division, St. Paul, Minn.). The particle was tagged with ^{57}Co, which is a gamma emitter with an energy of 120 keV and a half-life of 270 d. The particles were sized using a micrometer eyepiece on a light microscope (American Optical Corp.) to validate the manufacturer's specifications.

Measurement of Radiolabel Density in Free Polystyrene Particles

Fifty-five 1-ml suspensions containing ^{57}Co-labeled carbonized polystyrene particles of varying concentrations were made. Each suspension was counted in a well scintillation counter (Abbott Laboratories, Chicago, Ill.) with a sensitivity of 5×10^{-4} μCi for five 1-min counts. Two 20-μl aliquots were removed from each suspension and the particles were counted on a hemocytometer through a light microscope at 400× magnification. The original suspension minus 40 μl was recounted and the difference in counts represented the number of particles counted on the hemocytometer. This procedure was performed in triplicate for each suspension, generating 165 counting procedures. In addition, known (0.131 μCi and 0.121 μCi) sealed sources of radioactive ^{57}Co (New England Nuclear, Boston, Mass.) were counted along with each suspension. This procedure relates the cpm to number of particles and radioactivity in absolute microcuries. The relationship was graphed and designated the standard conversion curve.

Inhalation Procedure

The particles were dispersed in a solvent of 95% ethanol by vortexing and ultrasonication for 2 h prior to inhalation. This particle suspension was aerosolized from an air blast venturi-type nebulizer (Puritan-Bennett, Kansas City, Mo.) and connected via a three-way valve to the anesthetized, intubated sheep. The three-way valve had one port connected to the endotracheal tube, another to the nebulizer-syringe system, and the third port to an absolute filter. To inflate the lung with aerosol, a calibrated syringe was used to push 1 l of air through the nebulizer at a flow rate of 250 ml/s. A 6-s breath-hold was imposed by closing all ports, and the sheep returned to spontaneous tidal breathing by opening the port to the absolute filter. One breath (1 l) of aerosol was imposed each minute for 60–90 min.

Deposition and Clearance Scintillation Images

Lung images acquired by a gamma-scintillation camera (Dyna Camera 4/15, Picker Corp., Cleveland, Ohio) were stored and analyzed by computer (Nova 3, Data General Corp.). The camera was set to a photopeak of 120 keV at a window of 15%. A low-energy, parallel multihole collimator was used. While standing, the sheep was imaged from the dorsal aspect of the thorax, producing lung images with a resolution of 0.6 cm and a sensitivity of 2 μCi. The sites of ^{57}Co-tagged particles in a planar lung image were identified through image analysis computer programs that sum cpm while subtracting background counts and correcting for radioactive decay.

Lung Fixation and Slicing

The sheep were sacrificed to determine anatomical sites of particle deposition and number of radiolabeled particles in the lungs. Lung tissue was obtained by placing the anesthetized, intubated sheep on a respirator (Harvard Pump, Millis, Mass.) with a frequency of 20 breaths/min and a tidal volume of 300 ml. The jugular vein was catheterized and bled for 5 min and the thoracic cavity was opened. The right ventricle was opened and a catheter was inserted into the pulmonary artery. Freshly prepared fixative (polyethylene glycol 400, 95% ethanol, 40% formalin, and water in volumetric proportions of 10 : 5 : 2 : 3) was infused through the pulmonary vasculature at a flow rate of 120 ml/min for 10 min. The lungs were placed in the fixative for 12 h and air dried via a bronchial cannula at 30 cm H_2O pressure. The lungs were subsequently sliced into 50–65 4-mm-thick slices.

Quantitation of Particles and Radiation in the Lung

The fixed slices of lung were weighed and cut into segments. These segments were counted in the well counter along with a known ^{57}Co source. The cpm were translated into number of particles or microcuries from the standard conversion curve and the total numbers of particles in each slice were determined. Summation of all slices gave the total number of particles and amount of radiation (microcuries) deposited in the lung.

RESULTS

A point calibration was performed so that others may compare their gamma camera systems with ours, permitting the correlation of data and equations presented in this article to other systems. A point source of 500 μCi of ^{57}Co, 10 cm from the collimator, produced 1.97×10^5 cpm with a 15% window.

The number of radiolabeled particles counted on a hemocytometer was related to the amount of radiation (microcuries or cpm) measured by well scintillation counter. Figure 1 summarizes the relationship between the number of polystyrene particles and their radiolabel at various particle concentrations in terms of microcuries, cpm, and number of particles. All counts and microcuries were decay corrected to the date the particles were prepared and tagged with ^{57}Co. In this figure, a linear relationship between the variables by the method of least squares was found. This relationship has an r value of .98, $F = 3190$, and significance of $p < .001$. The linear relationship defines the standard conversion equations:

$$\text{cpm} = 0.231(\text{particles}) + 2.1 \times 10^4$$

$$\text{cpm} = 1.81 \times 10^6(\text{microcuries}) + 2.1 \times 10^4$$

Using five sheep containing different concentrations of radiolabeled particles, the numbers of particles or microcuries within the lung were determined by the procedure outlined in Fig. 2. The lungs were fixed, sliced, diced into sections, and counted in a well scintillation counter along with a known sealed source of ^{57}Co. The cpm for each

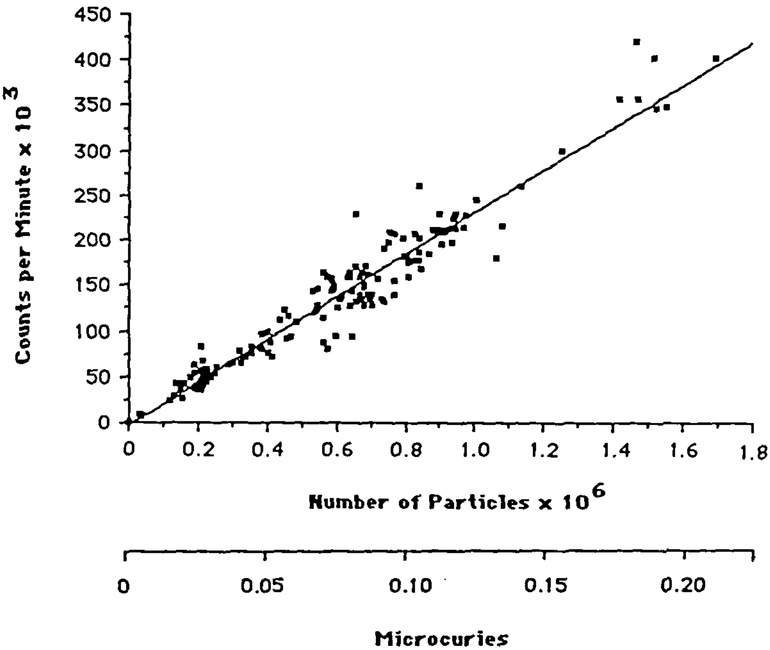

FIGURE 1. Relation between counts per minute measured by well scintillation counter and number of particles in saline suspension or microcuries of radiation in particle radiolabel (^{57}Co).

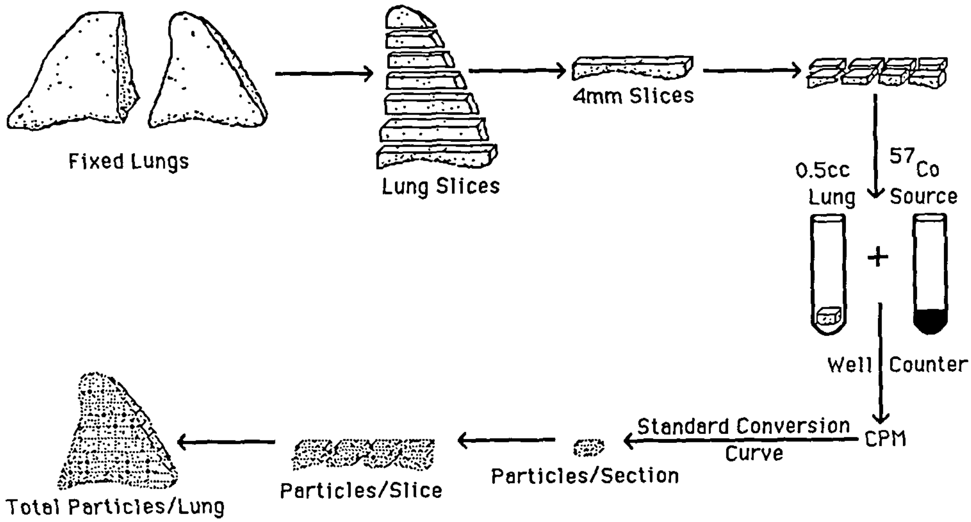

FIGURE 2. Determination of particles in lung from fixation, slicing, well scintillation counting, and standard conversion curve (from Fig. 1).

section was converted to particle number via the standard conversion curve (Fig. 1). All sections were summed into slices and all slices were summed into the whole lung. This procedure defined the relationship between cpm, microcuries, and number of particles in the whole lung counted by well scintillation counter, as linear by the method of least squares (r = .98) shown in Fig. 3.

The relationship between microcuries, cpm, or number of particles in fixed whole lungs acquired by gamma scintillation camera is shown in Fig. 4. The data by the method of least squares fit a linear relationship (r = .98). The ordinate in Fig. 4 is cpm from whole fixed lungs without an interposed thorax. Therefore, the radiation was attenuated only by the lung, without any intervening structures. The abscissa in microcuries or particles was defined by counting multiple fragments (Fig. 2) and summing for total content of microcuries or particles for each lung (Fig. 3). The number of particles or microcuries was then plotted versus the cpm previously acquired from the whole fixed lung.

The relationship between microcuries, cpm, and number of particles within the lungs of the intact sheep is also shown in Fig. 4. The ordinate represents the cpm acquired by positioning the camera over the posterior thorax of the live sheep. The abscissa is number of particles or microcuries in the fixed lungs as defined in Figs. 2 and 3. By the method of least squares a linear relationship between the variables was found with r = .98. The predictive equations from the data are

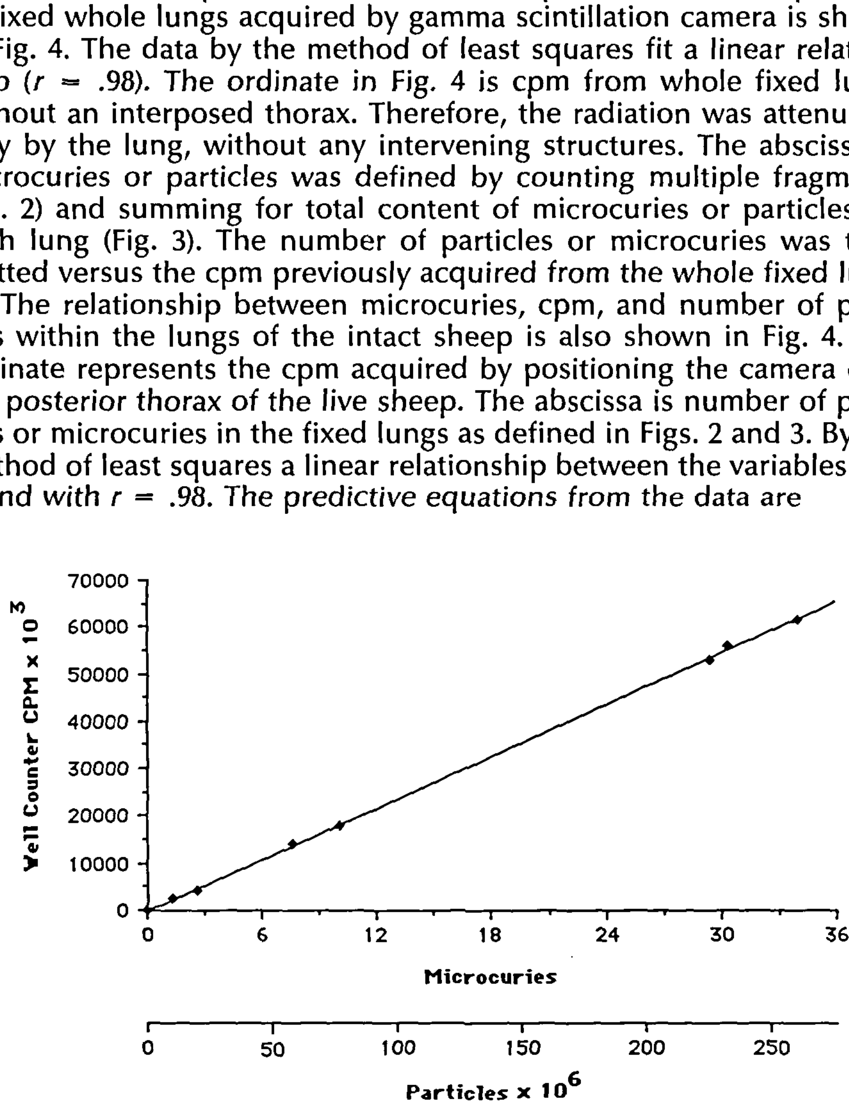

FIGURE 3. Total counts per minute from well scintillation counter of all summed tissue sections in each of seven fixed lungs, and number of particles or microcuries of radiation in particle radiolabel (^{57}Co) contained in the lungs.

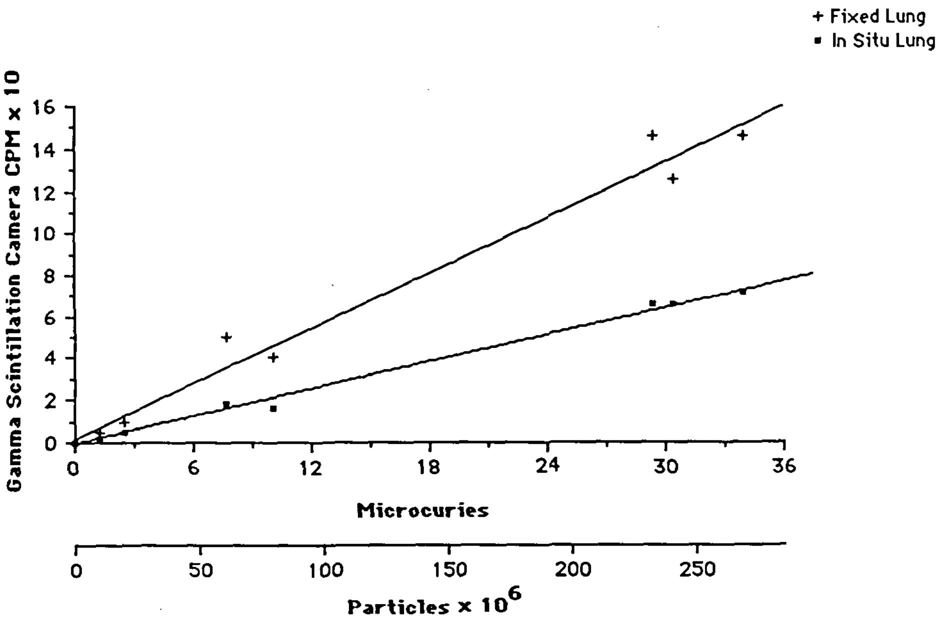

FIGURE 4. Counts per minute of the fixed lung (+) and of the lungs in the live sheep (■) acquired by gamma scintillation camera and number of particles or microcuries of radiation in particle radiolabel (^{57}Co) contained in the lungs.

$$\text{cpm} = 27.2 \times 10^{-6}(\text{particles}) - 64.6$$

$$\text{cpm} = 214.2(\text{microcuries}) - 64.6$$

The well scintillation counter and gamma-scintillation camera efficiencies were determined through a series of calculations. Sealed ^{57}Co sources, 0.131 μCi and 0.126 μCi, were counted with each particle suspension and lung section. The ^{57}Co source containing 0.131 μCi (1 μCi = 3.7×10^4 dps) produces 29.1×10^4 dpm. The actual cpm recorded by well counter was 23.5×10^4 cpm, determining a well counter efficiency of 81.0%. The ratio 0.131 μCi/23.5×10^4 cpm was used to calculate the total number of microcuries and dpm in whole lungs for Table 1 by the following equations:

$$\text{Total lung } \mu\text{Ci} = \text{total lung cpm} \times (0.131\ \mu\text{Ci})/23.5 \times 10^4\ \text{cpm})$$

$$\text{Total lung dpm} = \text{total lung } \mu\text{Ci} \times 37{,}000\ \text{dps} \times 60\ \text{s/min}$$

For example, the well scintillation counter total lung count for all sections of lung 2 was 55,830,511 cpm, which represented 31.1 μCi (6.9×10^7 dpm). The efficiency of a gamma scintillation camera is a function of a number of parameters: distribution of radioactivity in an organ, distance from camera, intervening tissue attenuating the radiation, collimator, and inherent sensitivity of the camera. The external counting efficiency of a system with all the above parameters either calculated or standardized was defined as the ratio of the cpm acquired from the live

TABLE 1. Relationship between Emitted Radiation in Intact, Resected, and Diced Lung

Lung number[a]	Body wt. (lb.)	Particle number/lung $\times 10^6$	Particle[b] number/g $\times 10^6$	Radiation (μCi/lung)	Ext. ct. eff[c] (particles/cpm, $\times 10^4$)	Resected lung[d] (μCi/cpm, $\times 10^{-3}$)	Lung in situ[e] (μCi/cpm, $\times 10^{-3}$)	Att. coeff.[f] (cm^{-1})	Coll. eff.[g] (cpm/dpm, $\times 10^{-5}$)
1	140	267.2	2.12	34.63	3.76	2.4	4.9	0.159	9.2
2	182	239.6	1.70	31.06	3.74	2.2	4.9	0.169	9.2
3	182	231.3	1.49	29.99	3.57	2.0	4.6	0.175	9.8
4	208	78.7	0.46	10.20	4.70	2.5	6.1	0.178	7.4
5	208	60.2	0.33	7.80	3.37	1.5	4.4	0.215	10.2
6	220	19.8	0.10	2.57	4.35	1.7	5.6	0.199	8.0
7	193	9.8	0.06	1.27	4.49	2.7	5.8	0.153	7.8
Mean					4.00	2.2	5.1	0.178	8.8
SD					0.51	0.4	0.7	0.022	1.1

[a]Either right or left sheep lung.
[b]Mean particles/g dry weight of fixed lung.
[c]External counting efficiency.
[d]Counts per minute acquired by gamma camera over posterior thorax of live animal.
[e]Counts per minute acquired by gamma camera over resected lung.
[f]Attenuation coefficient of chest wall.
[g]Gamma camera collecting efficiency.

animal to the number of particles or microcuries contained in the lungs. For example, from Table 1, a dorsal to ventral image of lung 2 from the live sheep contained 31.1 μCi and 239.6×10^6 particles produced 6398 cpm on gamma scintillation camera. The external counting efficiency of this lung was 3.7×10^4 particles/cpm and 4.9×10^{-3} μCi/cpm. The gamma camera and system collection efficiency was 0.0093% [6398 cpm/(6.9×10^7 dps) × 100]. Therefore about 1 count was recorded by the camera for every 11,000 disintegrations in the lung. These calculations were performed on seven lungs with the results presented in Table 1. The mean external counting efficiency was 4.0×10^4 particles/cpm and 5.1×10^{-3}, respectively. The mean collection efficiency was 0.0088% with a standard deviation of 0.0011%.

The linear attenuation coefficient u is described in the following equation (Prior, 1974):

$$N = N_0 \times e^{-ut}$$

where N_0 is the number of incident photons on a medium of thickness t and N is the number of emerging photons. The thickness of the chest wall of each animal was measured at autopsy and had variable thickness and tissue density. However, a mean thickness was determined for each animal and used to calculate an individual attenuation coefficient. This linear attenuation coefficient of the posterior thorax was measured by imaging the lungs in the live sheep and subsequently imaging the whole fixed lungs outside the thorax in the same orientation and distance from the collimated gamma camera as in the live sheep. The μCi/cpm values acquired for the lungs inside and outside the thorax are presented in Table 1. The ratios acquired by gamma-scintillation camera for lung 2 were 2.2×10^{-3} μCi/cpm from the resected lung without the intervening thorax and 4.9×10^{-3} μCi/cpm from the lung in the intact sheep. The mean wall thickness was 4.75 cm:

$$2.2 \times 10^{-3} = 4.9 \times 10^{-3} \times e^{-u(4.75\ \text{cm})}$$

$$u = 0.169/\text{cm}$$

The attenuation coefficients of the posterior chest wall for ^{57}Co at various concentrations in seven lungs are presented in Table 1. The chest wall had a mean attenuation coefficient of 0.178/cm and a standard deviation of 0.022/cm.

DISCUSSION

This study was designed to measure (1) radionuclide concentration in the lung in order to determine the lung load in terms of microcuries and particle number; (2) the attenuation coefficient of the mammalian posterior chest wall; (3) the external counting efficiency of an intact animal via gamma-scintillation camera in order to estimate radionuclide concentration and hence, radiolabeled particle concentration in the lungs; and (4) the collection efficiency of the gamma camera alone. These measurements established a set of standardized parameters for quantitation of radionuclide in the lung by noninvasive gamma-scintillation imagery in the live animal.

A considerable inaccuracy exists in quantitating the concentration of radionuclide in the lungs because of unknown attenuation losses and indirect methods in calculating inhaled radiolabeled aerosols. Our methods of direct visualization of the particles by light microscopy and determination of radionuclide concentration by well scintillation counting established a quantitative conversion equation (Fig. 1). Using this linear equation relating particles and microcuries to cpm, the distribution and absolute concentration of radionuclide in the lungs by summation of fixed lung sections (Fig. 2) was determined for each lung (Fig. 3). The totals were correlated to the gamma-scintillation camera lung image immediately before sacrifice and after fixation resulting in the linear relationships defined in Fig. 4. The equations derived from Fig. 4 were linear with an high correlation coefficient ($r = .98$), which defined the external counting equations (efficiency) under the standardized counting conditions. Thus, particle number or microcuries can be estimated directly and noninvasively from the lung images of the live sheep. We defined the external counting efficiency for our standarized system as ratios of cpm to particle number or microcuries (Table 1). Additionally, human and sheep have a similar lung size; the average dimensions of the sheep lung image are 46 pixels (1 pixel = 0.60 cm) apical to basal and 20 pixels lateral to medial, while the average human lung measures 44 pixels and 20 pixels, respectively. Thus, the sheep data may be of importance in calculating the number of rads absorbed during normal diagnostic procedures or inadvertent radionuclide exposures and inhalations of aerosolized particles in humans.

We found the overall ratio of cpm aquired by gamma camera to microcuries and particles in the in situ lung to be about 5.1×10^{-3} μCi/cpm and 4.00×10^{4} particles/cpm with coefficients of variation of 13%, over a range of 1.27–34.63 μCi and 9.8×10^{6} to 267.2×10^{6} particles, respectively. This suggests that the ratio of cpm acquired by gamma camera to the actual microcuries or particles within the lungs was relatively constant from sheep to sheep over a magnitude of change in radionuclide concentration. Thus, the counts recorded from a gamma camera lung image can be standardized to reflect an estimate of the microcuries or particles contained within the lung. In order to ensure the reproducibility of the external counting efficiency, a number of parameters must be standardized.

The camera face touched the posterior thoracic wall of the intact sheep, defining the distance of lungs to the NaI detector. The radionuclide was ^{57}Co (120 keV), detected by a wide field of view gamma scintillation camera with a low-energy parallel hole collimator. This established the gamma photon energy level and the collimation. The attenuation of the posterior thoracic wall was determined from the difference in cpm detected between the intact sheep lungs and fixed lungs (Fig. 4). This difference was used to calculate the linear attenuation coefficient expressed mathematically as $N = N_0e^{-ut}$ for each sheep lung. This equation usually applies to a medium of uniform thickness and density. The thoracic cavity presents a surface of varying thickness and densities which may fluctuate as fat, muscle, and bone content change with age, size, and weight. Because of these variations only an approximation of the thickness can be made. We used a mean chest wall thickness for each sheep measured at autopsy to calculate the linear attention coefficient using the data from Table 1. The cpm for the fixed lungs showed a greater variability than the lungs in the intact animal. This was unexpected in that we anticipated that the intact animal would have a greater counting variability due to the varying thickness and tissue density of the thoracic wall between animals. The mean and standard deviation of the attenuation coefficient were 0.178/cm and 0.022/cm, respectively, for all sheep. Thus, after the external parameters of distance, energy level, and collimation have been standarized the calculated attenuation coefficient did not vary greatly despite a magnitude of change in the radionuclide concentration from sheep to sheep.

In essence, there was a 9000 : 1 decrement in detectable radiation counts from lung tissue by well scintillation counter to the whole animal by gamma scintillation camera. This was due to the differences in geometry and sensitivity of the two detectors. However, the *r* values are greater than .98 for both detectors (Figs. 3 and 4). Differences in cpm acquired by gamma camera only are primarily due to attenuation by the posterior thorax and variability in lung size and density. However, the correlation within each series of measurements was excellent. For instance, all the points relating gamma camera images (cpm) to contained lung particles have a linear relationship over a magnitude of change in lung particle content (9.8×10^6 to 2.7×10^8) and particle distribution. The greatest particle density occurred upon deposition, when particles were found in the central airways as well as in alveoli. After particle clearance, the particle density decreased due to removal by tracheobronchial mucociliary action and macrophages; the remaining particles were primarily in the peripheral airways and alveoli. Thus, measurements performed after particle deposition or clearance must reflect anatomic differences in particle distribution. These differences did not demonstrate alterations in detectability of particles, since the relationship between cpm and particles over these ranges remained constant as shown in Fig. 4.

The efficiency of the gamma camera was also calculated for the varying concentrations of nuclide in the intact animal. The efficiency of the camera depends on a variety of parameters already discussed. These parameters were held constant for each sheep and the collection efficiency of our gamma scintillation camera is listed for each lung in Table 1. The mean percent efficiency was 8.8×10^{-3} of emitted dpm. Thus, the camera records about 1 in every 11,000 dpm emitting from the radionuclides within the lung.

The sheep was chosen because its lung size and anatomy are similar to those of humans. Lung images of humans and sheep have a comparable number of pixels apical to basal and lateral to medial. The areas ventilated by ^{127}Xe are also similar in humans and sheep. In addition to the similarity of size and ventilated areas, the number of generations from main bronchus to a terminal alveolus, O_2 consumption rate, lavage cellular composition, lung weight, and body weight (140–220 lb) compare favorably to humans (Leith, 1983). These favorable comparisons add support to the application of analogies to humans from conclusions drawn from the sheep data in regard to lung radiation dose, attenuation coefficient of the thorax, and external counting and collection efficiencies. We are uncertain regarding the assurance with which the collection efficiency of 1 cpm acquired by gamma camera for every 1.1×10^4 dpm in the sheep lung is transferable to humans. If the extrapolation is valid, it would permit an accurate measurement of retained radionuclides of the same energies in humans from clinical diagnostic tests or environmental exposures. The relatively small standard deviation found around this ratio, despite a range of variation in body size and lung radionuclide dose and distribution, suggests that these data may be extrapolated to humans; however, a firm conclusion would require further experimental support.

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