MECHANISMS OF PULMONARY CLEARANCE OF INHALED PLUTONIUM DIOXIDE AN AUTORADIOGRAPHIC STUDY AND PARTICLE SIZE ANALYSIS*

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Abstract—An autoradiographic technique is described for measuring particle size distributions of inhaled $^{239}PuO_2$ in alveoli of dogs and rats at various periods of time after exposure. Additional data are presented for the tracheo-bronchial tree of rats and lymphoid tissue of dogs. In rat alveoli, an early (6 hr) decrease in MMD is followed by a rise (2 days) and a second decrease (7 to 32 days). A similar cyclic change in CMD in dog alveoli is found with a different time sequence (1 to 406 days). The implications of these and other observations are discussed.

INTRODUCTION

The pharmacokinetics and toxicology of plutonium compounds have generated an increasing interest for over twenty years. Of particular interest has been the behavior of insoluble plutonium compounds after inhalation. Earlier studies by Langham, Abrams and others[1-5] have been extended with a view to relating potential human hazard with the properties of the material inhaled, with values for pulmonary deposition and retention and to the pathways and mechanisms of clearance.

In more recent studies in the rat[6-8] and dog, [9-13] one aspect of particular interest has been the relation of particle size to deposition and clearance of alveolar regions. The present report has utilized experimental material from rats in a collaborative study with Boecker[7] and from dogs in a study by Morrow and coworkers.[9-13] Although some pertinent aspects of the autoradiographic findings are included incidentally here, detailed reports of each study will appear separately. This paper deals primarily with the relation of particle size to pulmonary clearance.

Morrow and Casarett[9] reported that in dogs two total respiratory tract deposition patterns were observed, 56% deposition in one group exposed to aerosols with an average CMD of 0.24 μ, σ_g, 1.81 and the second group with an 88% mass deposition following exposure to aerosols with mean characteristics of CMD of 0.7μ, σ_g, 1.77. Studies by Bair *et al.*[10, 12] for three different count median diameters (0.60, 0.43 and 0.86μ) and presumably comparable particle distributions, further extended the relation of alveolar deposition and clearance to inhaled particle size.

To assess possible particle size dependency of rate and route of pulmonary clearance, Morrow and Casarett[9] utilized an autoradiographic estimation of particle size in tissue structures in a preliminary series of four dogs sacrificed from 16 to 125 days after exposure. Among other findings, they reported a more tenacious retention of small particles (0.04–0.12 μ diameter) in alveolar regions and, consequently a relative increase in the percentage of particles of this size found with increasing time after exposure. In an extensive study using nearly the same technique, Bair *et al.*[12] reported

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data which, although differing in some details and subject to differences in interpretation, were not markedly unlike that reported previously for equivalent inhaled particle sizes and approximately equivalent times after exposure.

Although the techniques used were reported to be the same in both studies, there were significant differences and both executions were subject to more imprecision than necessary. Neither report included attempts at characterizing the distribution as a continuous function and both used different particle intervals. The study by Bair and co-workers has the advantage of short time intervals after inhalation and, while not strictly comparable to the dog work reported herein and previously, is of considerable value in itself and of particular pertinence in this report in allowing a comparison with rat data for equivalent time periods after inhalation.

A correction for geometry was made in previous studies; Bair *et al*[12] apparently used the same correction of 40% efficiency for all track counts irrespective of the density of radial tracks arising from the particle. Morrow and Casarett[9] used a preliminary sliding scale based on a 30–40% efficiency. The present study employed a refined correction. Figure 1 illustrates the variable ease of detection of tracks. As the density of tracks increases there is a decreasing likelihood of viewing tracks. For example, with small numbers of tracks (A, B), it is relatively easy to detect tracks entering the emulsion at an angle to the horizontal. However, as the number of tracks increases (B, C), fewer of the angular tracks are detectable; thus a smaller fraction of the emissions is counted the larger the number of tracks associated with the autoradiographic manifestation of the particle.

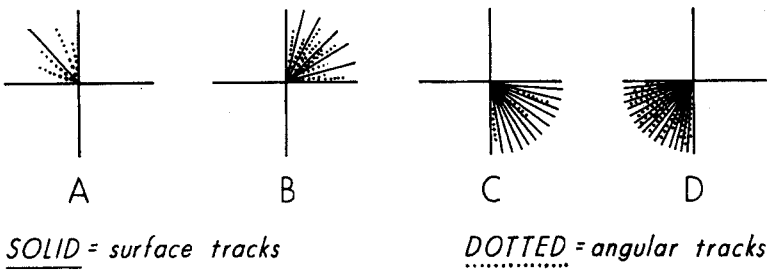

FIG. 1. Schematic representation of alpha track detectability. Relation of sunburst density to detection geometry.

METHODS

Animals were exposed, handled and sacrificed as previously described.[7, 9, 13] Tissues for autoradiography were fixed in 10% neutral buffered formalin, sectioned in paraffin at about 6 μ thickness and sections were floated on NTA* nuclear track plates (20 μ thickness) or covered with NTA nuclear track bulk emulsion (at least 20 μ thickness). Autoradiograms were stored for exposures of 1 day to 1 year and were processed under conditions specified by the manufacturer. Sections were routinely stained in hematoxylin and eosin.

* Eastman Kodak Company, Rochester, New York.

A curve for geometry correction was constructed. It will be noted in Fig. 2 that a measure of efficiency of detection can be taken as the detectability of tracks with the greatest angle of entrance into the emulsion and therefore the shortest apparent lateral length when viewed microscopically. Over 2000 sunbursts ranging from 10 to 285 readily observable tracks were examined for the track with the shortest apparent lateral traversal. As indicated in Fig. 2, the angle θ was calculated from the known length of track ("real") either by direct measurement or by calculation from the Pu energy and the stopping power of the emulsion. The

solid angle of the sphere which is, in fact, all that is really counted, can then be calculated and translated to an efficiency correction.

From autoradiograms of lung from each animal, random fields were viewed microscopically, a minimum of 500 particles was

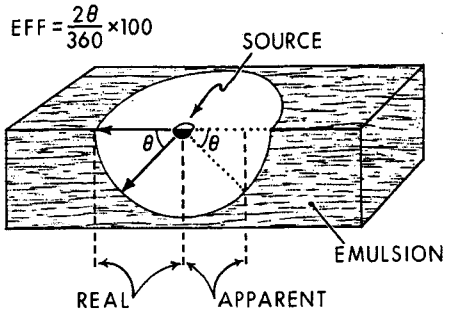

FIG. 2. Diagram illustrating calculation of track count efficiency. Geometry of particle track count.

counted for alveolar regions and the number of tracks for each was registered. Particles viewed in small, medium and large bronchiolar structures and in lymphoid tissue were also recorded. The total number of particles was about evenly divided among three autoradiographic exposures (short, intermediate and long). Each event recorded was corrected for geometry and exposure time and corrected to disintegrations per day. These values in turn were converted to particle size assuming a spherical shape, a density of 11.5 and a specific activity of 5.44×10^{-2} Ci/g PuO_2.

The particle size distribution thus obtained was divided into 15 intervals, interval 1 representing particles less than 0.0584 micron diameter and interval 15 containing all particles greater than 0.298 micron diameter. Data were placed in a program described by O. Raabe,[14] for calculation of the distribution parameters.

RESULTS

The calibration of efficiency of track count is shown in Fig. 3. It is clear that at low track density there is only a slight variation in efficiency, but as the track number density exceeds 100, there is a rapidly declining efficiency of detection (increased correction factor). It will be noted that assumption of a 5 μ depth of field in high density sunbursts, a not unreasonable figure, results in a theoretical efficiency which falls close to the empirically determined curve. It will also be noted that the maximal efficiency (smallest correction) is approximately 40% (2.50), the maximal value used in previous

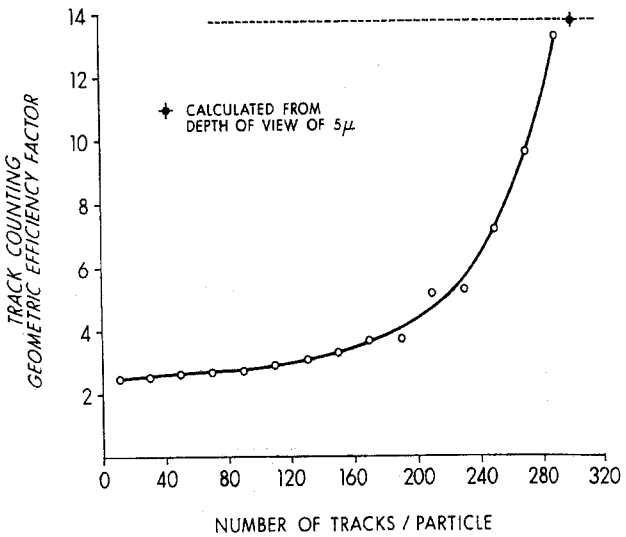

FIG. 3. Calibration curve of track counting efficiency correction.

studies by Morrow and Casarett[9] and Bair *et al.*[12]

Before the computerized data reduction became available, data were plotted and compared graphically. In Fig. 4 is shown an example of the particle size distributions obtained for alveolar regions of some of the rats from one exposure. ("Size" is presented as corrected raw track counts for a single autoradiographic exposure.) It appeared that the distribution first shifted to the left suggesting the clearance of smaller particles. Later the distribution appeared to move to the right of the line designating initially deposited material (immediate sacrifice) indicating the opposite, viz. that smaller particles were being retained preferentially. No significant changes in slopes could be discerned readily.

Although the progression of distribution shifts with time formed a pattern difficult to ignore, there remained a question about whether the distributions could all be considered to be part of the whole population. The more rigorous analysis of data provided several measures

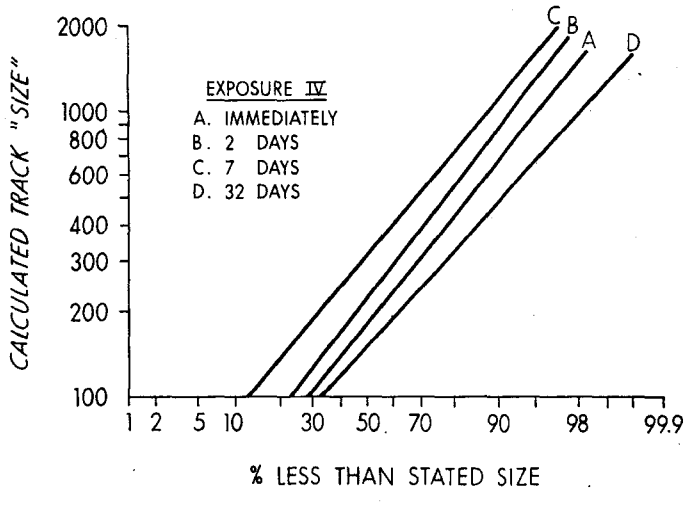

FIG. 4. Example of graphic treatment of particle size distribution of $^{239}PuO_2$ in rat alveolar regions.

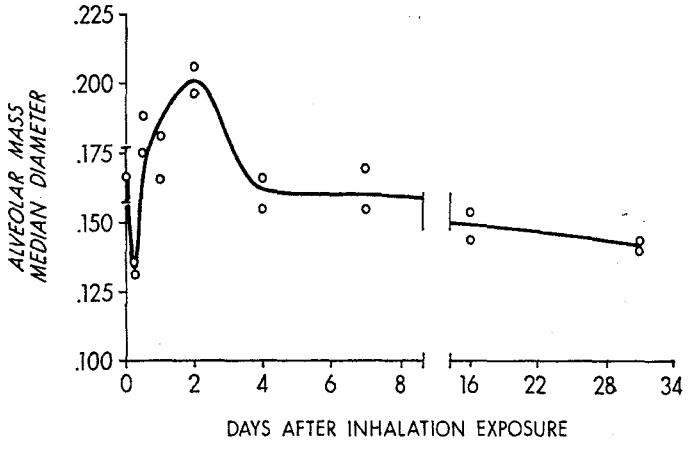

FIG. 5. $^{239}PuO_2$ particle size in alveolar regions of rat lung.

(CMD, MMD, arithmetic mean, etc.), but the most immediately meaningful change was in the CMD and MMD. Figure 5 is a plot of the MMD versus time after inhalation exposure for particles found in alveolar regions.

As suggested by previous graphic analysis, most of the particle distributions were statistically acceptable as log-normal ($p = 0.05$). The mean of counts from rats sacrificed immediately post-exposure is plotted at zero time and the range is indicated. There is apparently an early loss of larger particles as indicated by the fall in MMD at 6 hr, a return to significantly higher levels by 2 days after exposure and a gradual decline to a level below that found on immediate sacrifice at 32 days post-exposure.

Data from the tracheo-bronchial tree were treated similarly; the time change of MMD is presented in Fig. 6. As with the data from alveolar regions there is a marked decrease in median size until 1 day after exposure, an abrupt rise at 2 days and a more gradual decrease beyond 2 days leveling off at about 0.14 μ at 7–32 days post-exposure. Although

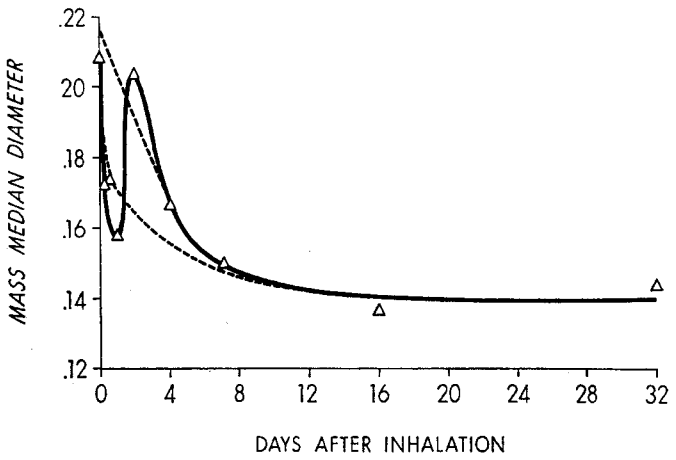

FIG. 6. $^{239}PuO_2$ particle size in tracheobronchial tree of rat lung.

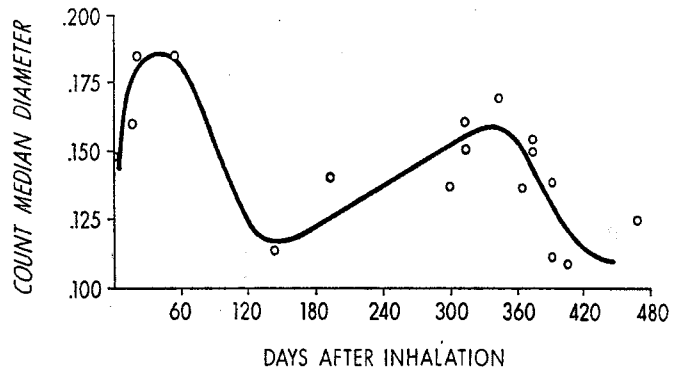

FIG. 7. $^{239}PuO_2$ particle size in alveolar regions of dog lung.

the data at 6 and 12 hr are quite consistent, two extrapolations have been entered (dashed lines) including and excluding these points. In either case the extrapolated value at zero time would be about 0.208–0.216 μ which corresponds to a CMD of approximately 0.16 μ.

Unlike the data from the short post-exposure periods in rats, alveolar particle distributions in dog lung fit log-normal distributions in only about one-fourth of the animals studied. The data are presented in the form of CMD versus days after exposure in Fig. 7. The CMD at early periods after exposure is approximately equivalent to that found for rats. Following an elevation at 22 and 56 days there is a marked fall by 125 days post-exposure. A second, gradual increase in CMD occurs between 125 and 375 days post-exposure followed by a second decrease beyond 375 days. No relation could be identified between the size distribution in alveolar regions and the inhaled aerosol characteristics or the onset or degree of pathologic changes as reported.[13] Insufficient numbers of particles in tracheobronchial areas of the sections prevented similar treatment of the data.

Examination of lymphoid tissue was hampered by sampling variability and comparatively small numbers of particles in the lymphoid tissue in the sections examined. The arithmetic mean particle diameter ranged from 0.084 μ to 0.211 μ with an overall arithmetic mean particle size of 0.160. With minor exception the fraction of particles in the upper intervals of the distribution ($> 0.22\ \mu$ diam.) was considerably smaller than that found in the alveolar particle distribution from the same dog. Additional sections of lymphoid tissue are being prepared to provide sufficient sample size for more meaningful treatment of these data.

DISCUSSION

It is readily apparent that the relation of particle size to rapidity of parenchymal clearance is not a simple one. It is equally apparent, however, that the technique described has a considerable potential for a continued, more refined attack on questions related to particle size and clearance. The material presented here is being extended in several ways. First, additional animals are available for similar treatment to provide data for other periods of time after inhalation exposure. The combination of autoradiographic exposure time is under scrutiny to establish the optimal exposure times and sampling methods to yield realistic results at the ends of the size distribution. Finally the treatment of the distributions as log-normal is convenient and fits most of the rat data and some of the dog data. However, a new program (O. Raabe[15]), tests the distributions against a general function and although more complex, is expected to supply more detailed information on the specific intervals of the distribution.

In the rat, the apparent shift of median diameter with time as viewed graphically (Fig. 4) is borne out qualitatively by the more exacting treatment of the data (Fig. 5). Alveolar deposition can be viewed as having resulted from a deposition of particles having a mean MMD of 0.167 μ and a mean geometric standard deviation of 1.44. This is different from the distribution of particles deposited in the tracheo-bronchial tree, which had a mean MMD of 0.208 μ and a mean σ_g of 1.48. Still higher is the MMD for alveolar deposition in the dog, 0.248, $\sigma_g = 1.51$.

The data obtained from rats point to an early (6 hr) loss of larger particles of the distribution followed by a preferential clearance of the smaller sizes (12 hr to 2 days). Although there are insufficient data to eliminate other pathways, the fact that the tracheo-bronchial clearance follows a pattern similar to alveolar clearance (with a time lag) strongly suggests that the major pathway during this period is the movement to the bronchiolar tree. This conclusion is further supported by the fact that the mass median for both distributions approach the same value of about 0.14 μ. The bicyclic phasing of particle size is similar to the qualitative description of tracheo-bronchial clearance of Po colloid[16] and is corroborative of apparent quantitative two-phase bronchiolar clearance described for Fe_2O_3.[17]

The alveolar clearance in the dog is more difficult to interpret partly because of the relative scarcity of points over long intervals of

time after exposure.* It would appear that there is a preferential clearance of small particles between 1 and 56 days roughly equivalent to that found in the rat at 6 hr to 2 days. Also similar to the rat data, except for the time element, is the decrease of the median to a level of about 0.14 μ at 125 days post-exposure.

For both series of animals, the increase in MMD suggests a possible alternative view, viz. that aggregation of particles occurred. On the basis of observations previously reported,[8,9] if aggregation is a factor during this period, it is most likely to be the result of intracellular incorporation by phagocytic elements. If this is true, it would follow that the fall in size could be related to the mobilization and removal of the phagocytic cells. In the rats the decrease between 2 and 7 days is consonant with the response of alveolar cells reported as a peak mitotic activity at 3 to 5 days after dust inhalation.[18, 19] No equivalent time periods or response data are available for the dog.

The prolonged increase in particle size in the dog from 125 to 343 days might also be considered to be due to aggregation. However, the lymph nodes, although increasing in concentration during this time,[13] show no significant changes in particle size distribution. Thus it is more likely that smaller particles are finding their way to the lymphoid tissue and that the slightly larger range of particles are remaining. There remains, of course, the distinct possibility that some particles are being transported to lymphoid tissue while others of equivalent initial size are more subject to aggregation in alveolar regions with the increased residence time.

The decrease in size occurring at about one year after deposition is also unaccompanied by any significant increase in large particles in lymphoid tissue in the limited data available. Further, although it was expected that changes at this time period might be related to pathologic changes, no clear relationship could be identified. Unless further cycling could be expected, the particle size at the end of the series might be approaching a "steady state" size at very long periods following exposure. It is of some interest that Wurm and Einbrodt[20] using electronmicroscopy, have recently reported CMD's for mineral in miner's lungs below 0.08 μ for periods of 5 to 14 years after exposure. From the dog data a reasonable terminal size is about 0.11 at about 400 days after exposure. These two figures are remarkably similar for having been obtained for different materials in different species and by different methods. Other work[21, 22] suggests that particle size in human lung remains relatively constant over long periods of time at about 0.5 μ as determined by essentially gravimetric methods.

With regard to the implications of particle size in dose distribution, it is essentially immaterial whether aggregation occurs; the fact remains that there is a variation of the size of the sources with time and that it is a complex function, perhaps cyclic for the earlier intervals after exposure. Although the absolute differences in median size are not great, these parameters, translated into total number or mass in the tissue might be expected to be a factor in dose-response relations. Some differences in effects of inhaled PuO_2 have been reported between studies at Hanford[23, 24] and Rochester.[13] Morrow *et al.*[13] have pointed out that these differences might well be related to dose rate differences as calculated from deposition measurements and rates of clearance. It should be added that, at the level of microdistribution of dose, there are also dose rate differences depending upon particle size differences whether in the inhaled atmosphere or, as illustrated here, in the patterns of change occurring in the animals at various time periods after inhalation. The marked lymphopenia observed[13, 24] directs attention to a particular need for the application of this technique to distribution of dose to lymphoid tissue.

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* Additional data are being obtained on dogs sacrificed between 56 and 125 days after inhalation. A collaborative study with Drs. Bair and Park of Battelle Northwest has been initiated for time periods up to about 30 days post-inhalation.

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