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PAPER NO. 3

PARTICLE DEPOSITION AND CLEARANCE AS A TEST OF TOXIC EFFECT*

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INTRODUCTION

The field of inhalation toxicology is currently in an intriguing active phase characterized by two features: (1) compilation, description and quantitation of effects other than mortality that include physiological, morphological and biochemical parameters, and (2) development of an understanding of biologic responses in terms of the physical and chemical properties of inhaled materials. Further development in both of these areas seems necessary for inhalation toxicology to become a predictive discipline as opposed to merely a descriptive one. The phenomena associated with deposition and clearance of inhaled particles have recently been recognized as important aspects of the defensive mechanisms of the lung. The purpose of this paper is to justify the use of deposition and clearance tests in the inhalation toxicology laboratory.

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** Affiliated with the Neutron Generator Facility, San Diego State University, San Diego, California, 92182.

Particles, or aerosols as they are called when airborne, can be broadly classified with respect to their origin. Naturally occurring aerosols include spores, pollens, microorganisms, inorganic dusts, ash, degradation products of various plants, liquid condensation droplets and various solids formed from naturally occurring volatile materials. In and about areas populated by humans additional aerosols include those produced from combustion of fuels, a multitude of industrial processes, erosion of machinery and building and household materials, spraying devices, and again, reactions of gaseous materials. Aerosols in and about localized workplaces form another practically innumerable subcategory. A common belief, probably justifiable, is that virtually all aerosols are capable of producing toxic responses in humans if inhaled in sufficient concentrations over a sufficient time (Christie, 1967).

For purposes of scientific elucidation, the fate of many inhaled aerosols can be analyzed into two phases: (1) deposition on surfaces of the respiratory tract, and (2) clearance (or lack of) after deposition has occurred.

The patterns of deposition of inhaled aerosols are becoming understood in terms of forces that act on airborne particles, the air flow characteristics of breathing, and the geometric properties (morphology) of the respiratory tract. Mammalian respiratory systems have geometric and air flow properties such that particles within a given range of size and shape tend to deposit preferentially in characteristic locations. For example, the human nose is known to be highly efficient in collecting particles with aerodynamic diameters greater than a few micrometers. The deep lung can only collect particles that have eluded the nose (or mouth) and the tracheobronchial tree; that is, particles with aerodynamic diameters below a few microns.

It appears that clearance mechanisms at various levels in the respiratory tract are efficient in handling particles in the size ranges that preferentially deposit at a given site. For example, alveolar macrophages seem to exhibit efficient engulfment for particles in the one micron diameter size range; this is just in the size range of high deposition probability in alveoli. Infectious organisms are often in the size range for deposition in the deep lung, where conditions are usually favorable for their rapid reproduction. Fortunately, macrophages can inactivate many infectious organisms. Similarly, the nose effectively clears the largest inhalable particles via sneezing, blowing and mucus movement, and the moving mucus of the tracheobronchial

tree is efficient in transporting large quantities of solid or liquid particles of various sizes, shapes and densities. The point is that deposition and clearance phenomena appear to be somewhat matched or balanced with respect to particle size characteristics. This being the case, one might suspect that alterations in either the deposition or clearance patterns could predispose one toward future injury from inhaled aerosols. In some instances, of course, shifts in deposition or clearance patterns might act to afford increased protection.

Fortunately, despite differences in size and morphology, most animals appear to have clearance phenomena that are remarkably similar with respect to rates and mechanism; for example, they commonly have mucociliary clearance in the nose and tracheobronchial tree and a macrophage response in the alveolar spaces. Also, though correspondence is not as close here, basic similarities exist in aerosol deposition characteristics and in responses to toxic materials. Thus, it is reasonable to consider tests of aerosol deposition and clearance in animals in toxicologic evaluations of injury.

INHALED AGENTS THAT ALTER DEPOSITION OR CLEARANCE PATTERNS

Several materials are known to alter deposition or clearance (including killing or inactivation of microorganisms) of inhaled particles. A few examples will serve to illustrate. Cigarette smoke, an almost ever-present co-insult in human inhalation exposure situations, has understandably been well studied. The controlled studies of Albert et al. (1969, 1970, 1974) show effects in humans and donkeys that depend on dose and exposure time. Low single doses or early effects of repeated exposure to smoke were associated with acceleration of clearance rates in the tracheobronchial tree of both species. Heavier doses and long-term repeated exposures were associated with sporadic clearance, intervals of clearance stasis, and even retrograde movement of deposited particles (again in both species). Cigarette smoke exposures (220 mg/m^3) have been shown to increase deposition and delay clearance in rats (Garver, 1968) and to increase the survival of inhaled viable bacteria in hamsters (Henry et al., 1970). In the hamster study, excess deaths due to bacterial infections were seen in animals exposed to cigarette smoke for 2 hours ("3% v/v").

Preexisting influenza infection has been shown to impair both upper and lower respiratory tract clearance. Studies by Green (1965) with P-8 virus infected mice that were exposed to viable staphyloccocus bacteria showed that infected animals did not effectively kill the bacteria. Similarly, Creasia et al. (1973) found that P-8 virus-infected mice had drastically impaired clearance of radioactive "insoluble" particles. In humans, Camner (1973) found that influenza infection could impair tracheobronchial clearance for up to one month after disappearance of the familiar clinical symptoms.

Elliot Goldstein and coworkers (1971, 1974) reported work in which mice were challenged with radiolabeled viable staphyloccus both before and after exposures to relatively low levels of ozone and nitrogen dioxide. Prior exposure to ozone (0.6-2 ppm, 17 hours) or ozone plus nitrogen dioxide (0.1-0.1 and 1.5-4.2 ppm, 17 hours) lead to: (1) decreased overall deposition of bacteria, and (2) impaired killing of deposited bacteria. In the same series of studies, exposures of ozone plus nitrogen dioxide (0.4 and 4-6.8 ppm, 4 hours) after inhalation of bacteria, caused increased survival of the bacteria. Ozone alone at 2 ppm (4 hours) was observed to cause increased survival and increased clearance of the inhaled bacteria.

Sulfuric acid mist exposures by Fairchild et al. (1975) at 3 mg/m^3 (1.8 μm CMD) in guinea pigs caused increased total deposition of inhaled streptoccocus.

Sulfur dioxide (1 ppm, 7 hours, 5 days to 25 days) has been shown to diminish the clearance of inert particles in both the lung and tracheobronchial tree in the rat by Ferin and Leach (1973). A similar effect was seen in donkeys after brief exposure (300 ppm SO_2, 30 min) by Spiegelman et al. (1968).

Many other agents have been reported to change deposition and/or clearance patterns in various species.

EXPERIMENTAL DESIGN FOR DEPOSITION AND CLEARANCE TESTING

A successful test of deposition and clearance implies that certain criteria have been met. These criteria apply to the test aerosol, animal subjects, experimental plan, and analysis of data. Recommendations can be made in each of these areas.

The Aerosol

For deposition and clearance studies, the aerosol should be inhaled by the subjects and have an aerodynamic size that permits significant deposition beyond the nose. For most studies an aerosol smaller than about 5 μm in diameter is appropriate. Monodisperse aerosols, or at least those that have diameters distributed with geometric standard deviations less than about 1.3, should be used. Aerosol characteristics should be reproducible from one run to the next. The aerosol should be detectable in the lung, either by external radiation detection or chemical/biological assay in excised lung. Radioactive aerosols should have a tag that does not have excessive leaching; a few percent per day in the lung environment is sufficient. Initial activity of labeled aerosol should be on the order of one microcurie to allow for precise external counting. An aerosol that fits the above criteria is radiolabeled polystyrene-latex. The basic particles are available commercially (Dow Chemical Company, Midland, Michigan) in several sizes, and methods for labeling with radioisotopes are available (Szende et al., 1975 and Black and Walsh, 1970).

The Animal

Unanesthetized animals should be used when possible in order to avoid variable and often uncertain effects associated with anesthesia. In most studies two species should be used, especially when one is either the guinea pig or the rat. Rats tend to have respiratory infections and guinea pigs appear to have unusually reactive airway musculature. Individual subjects should serve as their own controls to reduce variability in the data. Healthy individuals usually have relatively repeatable deposition and clearance phenomena, but variation within a group may be large.

Experimental Plan

Exposure to the aerosol should be to the nose or mouth to avoid deposition of large amounts on fur or skin. Deposition of material on fur of laboratory animals can interfere with subsequent assay of amounts in lung and can lead to ingestion of large quantities of particles. A determination of the initial deposited amount should be made immediately after exposure (within minutes), and the inhalation exposure should not last more than about 20-30 minutes. Long inhalation exposures are complicated by concurrent clearance occurring during deposition.

Amount in the lung should be determined with sufficient frequency to define the clearance curve. Ideally, the amount in the lung should be quantitated hourly for the first few hours and daily for several days. It is important that clearance be followed for long enough to properly define the clearance curve. The tracheobronchial tree is usually cleared by about 1-2 days but deep lung clearance can require several days, even months or years, for highly insoluble materials.

Data Handling

To determine the effect of toxic agents on clearance, statistical tests should be employed. This necessitates reducing clearance curves to numerical values; the fewer parameters used to define the curve, the simpler the tests will be. Figure 1 depicts three out of the many ways of quantitating clearance curves: (1) analysis into exponential components; (2) fitting with a polynomial function; and (3) calculation of moments. Each method yields numerical values that can be given mean values and standard deviations for a group of observations. Statistical tests for significance can then be performed on these values, permitting one to demonstrate significant changes in clearance patterns (Figure 2).

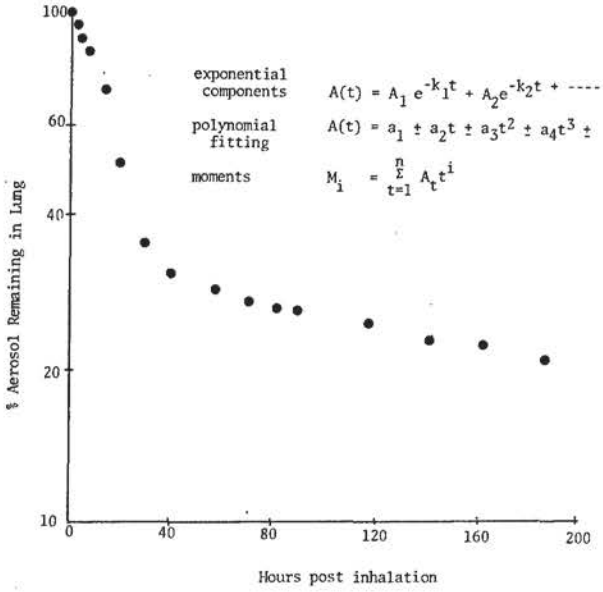

Figure 1. Three methods of reducing a clearance curve to quantitate parameters that can be used in statistical testing. The method that gives fewer parameters for testing would in general be more sensitive for detecting differences between groups.

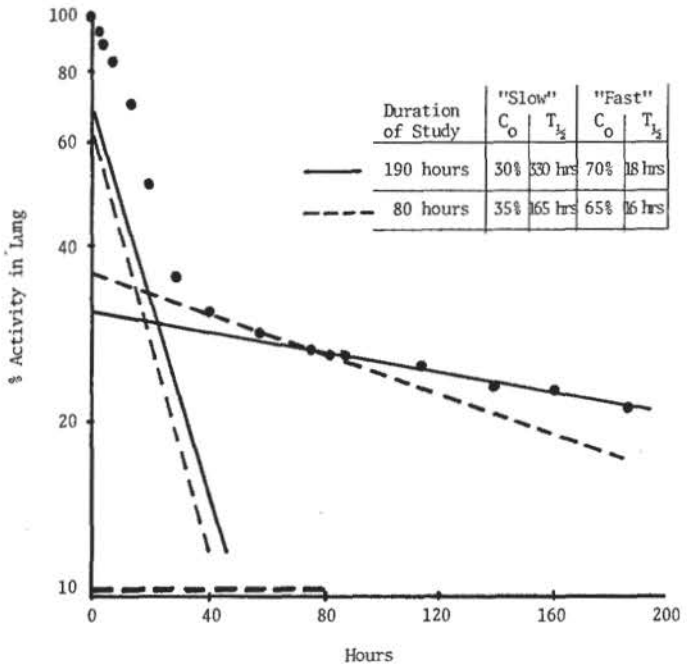

Figure 2. Demonstration that the duration of a clearance study has an effect on the exponential components of a clearance curve. Had this study been stopped at 80 hours different component curves would have resulted.

EFFECT OF CIGARETTE SMOKE EXPOSURE ON PARTICLE CLEARANCE IN THE RAT

Data resulting from a modest study will be presented to demonstrate the effect of a toxic agent on tracheobronchial clearance. Rats were used since they were inexpensive and easy to handle. Eight Sprague Dawley rats were briefly exposed, nose only, to a radioactive silver aerosol. The animals were then divided into two groups: one group was exposed to cigarette smoke for four hours, the other group breathed ordinary air and served as a clearance control. Clearance curves were determined for each animal by measuring the radioactivity in the thorax every 100 minutes for the first day, every 200 minutes the second day, and less frequently for about eight days. These measurements were made by using a collimated gamma-ray detector placed above an opening in a lead shield. The animals were placed beneath this collimator shield such that only gamma rays emitted from the thoracic region were measured. Feces were collected every time a thoracic count was made, and the radioactivity in each sample was determined.

Aerosol Exposure

An exploding wire aerosol generator (a 4 microfarad capacitor charged to 6.5 kilovolts) of the type described by Karioris and Fish (1962) was used to aerosolize 20 cm of 0.005 inch diameter silver wire. The wire had previously been neutron irradiated and had an induced activity of about 0.75 microcuries of ^{110m}Ag (beta and gamma, 250 day half-life) per cm of wire. The aerosol had spherical primary particles that were distributed approximately log-normally with a count median diameter of 0.07 microns and a geometric standard deviation of 1.6. Electron micrographs indicated that most of the aerosol was in the form of agglomerates of primary particles with an aerodynamic median diameter of about 1 μm when inhaled by the animals. The radioactive wire was exploded inside a 22 liter exposure chamber, five minutes were allowed for settling of large particulates, and the animals were exposed nose-only to the aerosol through ports in the chamber walls (Figure 3). During the actual explosion, rats were held in a separate room to isolate them from the loud noise. The exposure time was 15 minutes and the mean initial lung deposition was estimated (using wheat-filled phantoms) to be about 0.1 microcuries, or about 0.2 milligrams of silver. No anesthetics were used as the animals were docile and cooperative during both the aerosol exposure and the thoracic counting procedures.

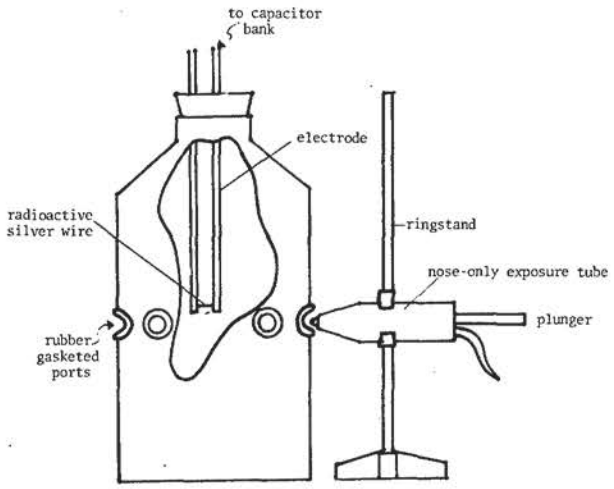

Figure 3. Aerosol exposure set up for simultaneous nose-only exposure of 8 rats to radioactive silver particles.

Smoke Exposure

The experimental group was exposed to fresh tobacco smoke generated by a machine which drew air continuously through several lit unfiltered cigarettes and gently blew the smoke into a large box (one cubic meter volume). The animals were placed inside cages within this box 30 minutes after exposure to the silver aerosol. They remained in the smoke for four consecutive hours, being removed only twice for two minutes each time, for thoracic activity measurements. The smoke concentration was maintained at a level such that taking a breath inside the smoke chamber (smoke exposure box) gave the experimenters the same subjective experience as inhaling during normal cigarette smoking.

Clearance Measurement

The animals were placed in plastic restrainers beneath a NaI(Tl) crystal (3" diameter) for gamma activity determinations of their thoracic regions. Two inches of lead were used to shield the head and gastrointestinal tract from the gamma detector. The shield, with a 2-1/2" wide opening above the thoracic area, had been designed using roentgenograms of all of the rats so that ^{110m}Ag in either the head, stomach or intestines was shielded from the detector.

Results

Longitudinal body scans of radioactivity indicated high initial activities in the head region, and it was feared that the fur on the head had been contaminated with significant amounts of ^{110m}Ag. However, this activity declined rapidly indicating a fairly clean nose-only exposure and that the initial activity was probably due to high deposition inside the nose and throat.

When compared to the air breathing group, the animals in the smoke-filled chamber were less active when in their cages, preferring to sit quietly, but they resisted handling by struggling considerably more than the control animals.

Clearance curves for control and smoke-exposed groups (Figures 4 and 5) were arrived at by averaging the values of all animals in each group. Cumulative activity excreted in the feces (Figure 6) is shown for both groups and is in terms of percent of total activity excreted during the data collection period.

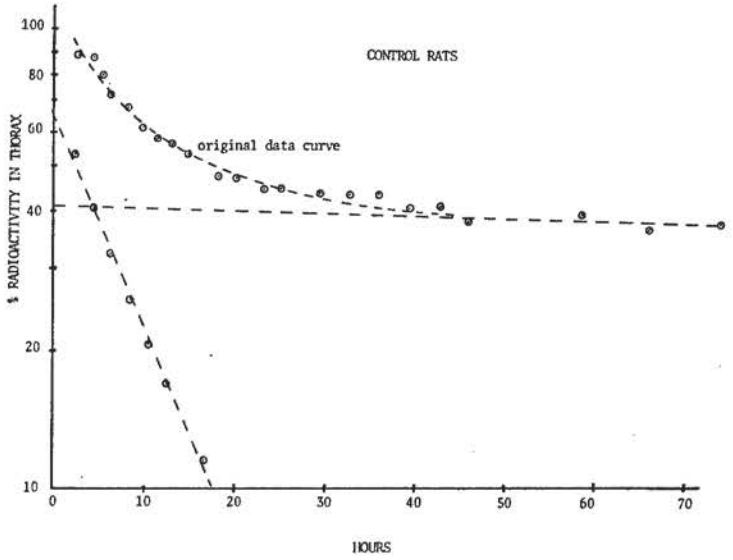

Figure 4. Thoracic clearance curves for control rats. Exponential components, "short" and "long" term, are shown.

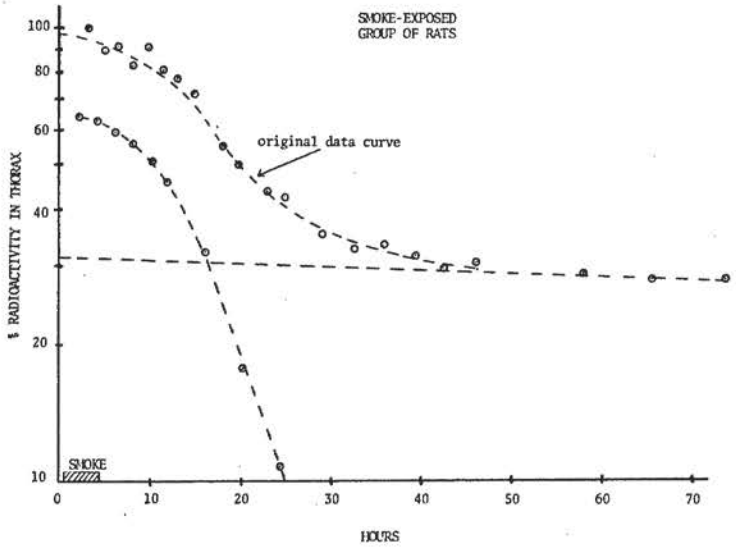

Figure 5. Thoracic clearance curves for rats exposed to cigarette smoke. Exponential components, "short" and "long" term, are shown.

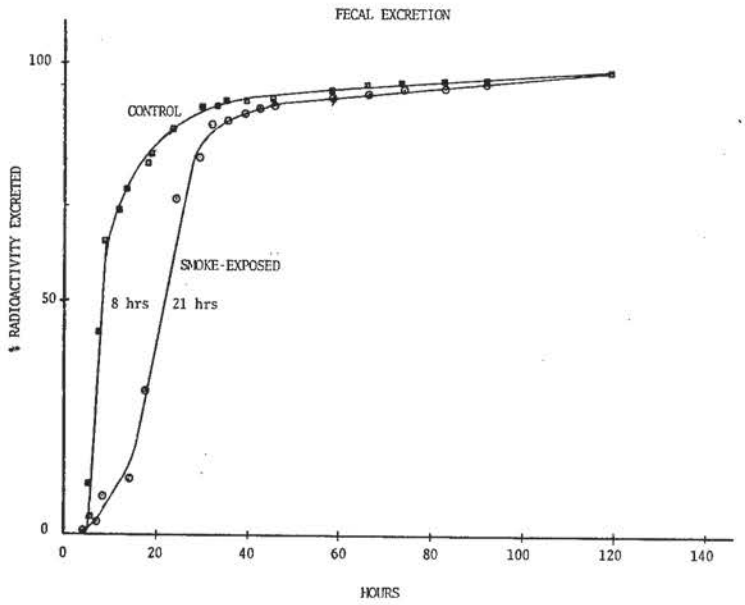

Figure 6. Cumulative fecal excretion of inhaled radioactivity for control and cigarette smoke-exposed rats. Half of the activity was excreted in the first 8 hours by the control group and in the first 21 hours by the smoke-exposed group.

The thoracic clearance curves appear linear (on a semi-log plot) after about 40 hours. This linear portion has a half-life of about 285 hours, with no significant difference between the two groups. Extrapolating this linear curve toward zero time and subtracting it from the original clearance curve produces a "short-term" clearance curve. For rats, this curve is usually also linear on a semi-log plot and is often assumed to represent mucociliary clearance of particles deposited on the ciliated portions of the respiratory tract. The control group's short-term curve is linear over its entire range and has a half-life of 6-1/2 hours. The smoke-exposed group's short-term curve does not appear linear until 12 hours after the animals were removed from the smoke chamber. The linear portion of this curve has a half-life of 6 hours which is not significantly different from the control value.

The time at which cumulative fecal excretion of ^{110m}Ag reached 50% of the total excreted was 8 hours for the control group and 21 hours for the smoke-exposed group. The difference, 12 hours, is almost identical to the period of time during which thoracic clearance was depressed

in the smoke group. This lag in excretion was not due to fecal retention by the smoke group since both groups produced fecal pellets at the same rate throughout the experimental period. It is, therefore, concluded that the brief exposure to cigarette smoke blocked movement of silver from the respiratory tract to the gastrointestinal tract, and that this block was effective for 12 hours after the smoke exposure terminated.

CONCLUSION

The status of deposition and clearance phenomena is an important consideration in inhalation toxicology. The techniques of aerosol challenge have been recently developed to a sufficient degree that routine testing of deposition and clearance of inhaled particles is now feasible in the toxicology laboratory.

ACKNOWLEDGEMENT

Dr. L. L. Skolil of the Physics Department, San Diego State University, encouraged and aided the original experimental work reported in the section titled "Effect of Cigarette Smoke Exposure on Particle Clearance in the Rat."

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