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UPTAKE AND FATE OF INHALED PARTICLES AND GASES: THE IMPORTANCE OF SPECIES DIFFERENCES

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INTRODUCTION

Dr. Whittenberger may be right that comparative physiology has been around for a long time, but the challenge of describing how different species respond to inhaled toxic agents is not a trivial task. Rather, we have been asked to discuss a central problem in toxicology: How do we interpret animal experiments to better predict the potential of an agent to cause damage in humans? In other words, how do we extrapolate from one species to another and from animals to humans?

Although many species have been used to assess the toxicity of chemicals, and although species differences are both recognized and argued about, we lack a complete and systematic description of the differences among commonly used laboratory animals. The subspecialty of inhalation toxicology is no exception. It is difficult to abstract a comprehensive description of species differences from the literature because so many different kinds of animals and aerosols have been used in various combinations. Several theoretical and experimental contributions exist, but the problem is far from solved. At least three aspects of exposure to toxic particles and gases should be considered. They are: deposition, clearance, and the magnitude and type of biological response.

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DEPOSITION

Palm and co-workers (1956) studied retention of dust in the lungs of guinea pigs and small monkeys; the percentage of alveolar retention as a function of particle size was substantially the same as that found earlier in humans. Friedlander (1964) used dimensional analysis to investigate deposition of particles in the lower lung. Kliment (1973) identified several of the important dimensionless groups of physiologic variables that appear in aerosol deposition problems and predicted the degree to which these groups of variables are the same in rats, guinea pigs, rabbits, and humans. More recently, Stauffer (1975) has used dimensional analysis to predict that the probability of deposition of inhaled aerosols should be the same for different animals in the case of sedimentation or turbulence-dominated deposition. The predictions of McMahon et al. (1977) differ from those of Stauffer (1975), and suggest that diffusion-dominated deposition is also independent of body weight.

McMahon et al. (1977) attempted to specify each of the physical mechanisms of particle deposition by identifying the controlling dimensionless group. For example, in the particle impaction problem, the collection efficiency, E (the percentage of particles entering that is actually deposited), increases as the Stokes' number, St, increases. The Stokes' number is the ratio of the stop distance of a particle to the characteristic dimension of the system. Similarly, the Froude Number, y, which is the ratio of the particle sedimentation velocity and the through-flow velocity, governs the sedimentation of particles in the nose, pharynx, and large airways during quiet breathing. Diffusion of small particles to the walls of the alveoli increases when the dimensionless diffusion time, DT/a^2, increases. D is the diffusion coefficient (which depends directly on temperature and inversely on air viscosity and particle radius), T is the breath period, and a is the alveolar diameter.

If all of the dimensionless groups varied with body weight, understanding the scaling rules for deposition of particles in the lungs would be complicated and difficult. Fortunately, several of the dimensionless groups are independent of body weight. Although many important physiologic parameters, such as breath period and ventilation, change as body weight changes, competing effects sometimes cancel one another in the controlling dimensionless groups. Then the collection efficiency is independent of body size for the same aerosol size.

McMahon and co-workers (1977) tested these ideas with a "Noah's Ark" approach. They simultaneously exposed 6 different species to the same 0.78μm aerosol of gold-198 and compared both total deposition and site of deposition among species. When the total amount of aerosol deposited was divided by the animal's

body weight, it was found that the smaller animals received more particles/gm than the larger ones. However, as expected, the collection efficiencies (the fraction of inhaled aerosol deposited) for both the lungs alone and the lung, nose, pharynx, and airways combined were substantially independent of body size.

So far, we have discussed deposition in relation to normal breathing patterns at rest. I now want to remind you that many of the things we do as toxicologists will affect the breathing pattern. The breathing pattern in turn will affect the dose of particles or gases retained by the animal. When you restrain an animal and give a head-only exposure, the breathing pattern changes. Generally, the animal will breathe faster and more shallowly. Exposures to irritant gases, such as ozone, will do the same thing; they also generally result in a shallower and more rapid ventilation. These changes in breathing pattern can have a profound effect on the lung dose.

Recently, Valberg et al. (1982) have described how breathing pattern can influence deposition sites of an inhaled ^{99m}Tc-labeled aerosol. First, we found that the collection efficiency varied from 2% to 40% as we explored a variety of breathing patterns. Not only did the collection efficiency (the fraction of inhaled aerosol deposited) change, but there were also significant changes in the distribution of retained aerosol. After the aerosol exposure the lungs were inflated, dried in a microwave oven, and sliced (see Figure 1).

Figure 1. A slice of dog lung exposed to a submicronic ^{99m}Tc-labeled aerosol. After aerosol exposure, the lungs were fully inflated to 35 cm H_2O pressure and dried in a microwave oven. The rigid lungs were then sliced at 1 cm intervals.

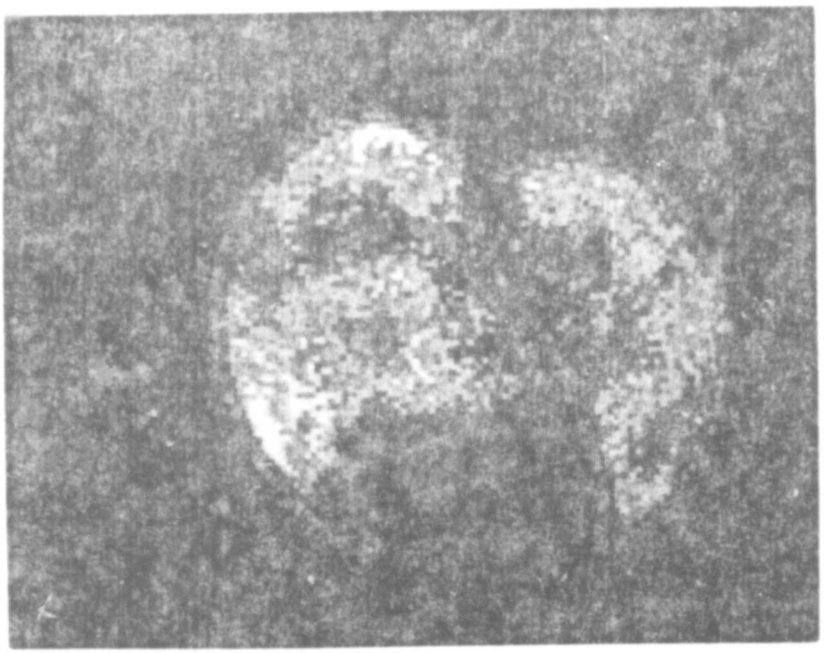

Figure 2. Slices prepared as described in Figure 1 were placed on a gamma camera with a high-resolution collimator and interfaced with a computer. Shown here is a photograph of the cathode ray tube out-put of the gamma camera showing the distribution of radioactivity over the slices of dried dog lungs. Each picture is a matrix of 128 x 128 cells that shows the activity with white and orange as high activity and blue and black as low and no activity. The lungs shown here were ventilated with a large tidal volume and a low breathing frequency. Note that the aerosol distribution through the parenchyma is relatively uniform but the airways are relatively free of retained particles.

You can easily identify the pleural surface, pulmonary parenchyma, and large and small airways. Then we used a number of techniques to look at the distribution of retained aerosol in such a slice. You can take this slice and lay it on the face of a gamma camera. With a slow deep pattern of breathing, the amount of aerosol deposited in the airways is relatively less than that in the parenchyma (Figure 2). With a rapid, shallow breathing pattern, there is much more deposition in airways than there is in the cooler parenchyma (Figure 3).

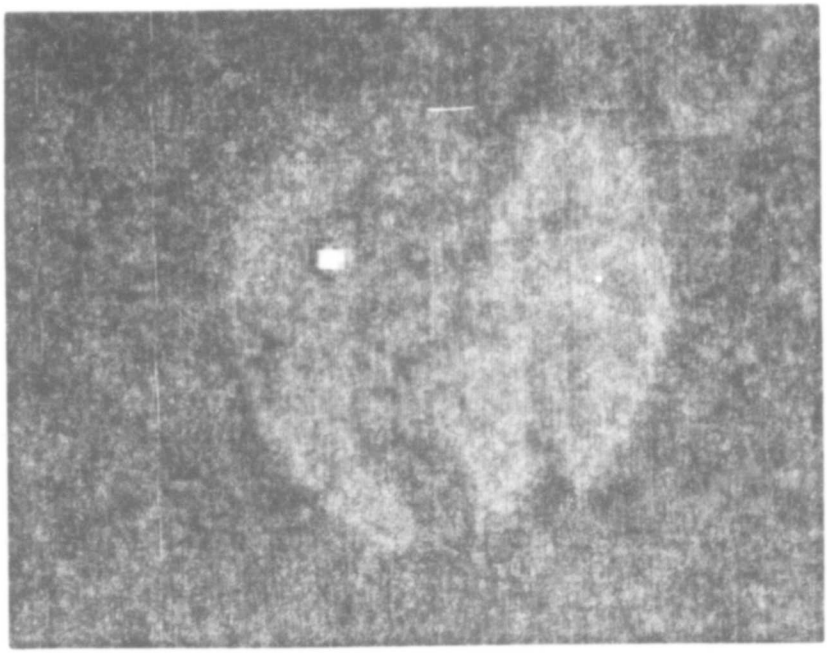

Figure 3. The slices shown here were prepared as described in Figures 1 and 2, but the lungs were ventilated with a small tidal volume and at a high breathing frequency. Even though the sub-micronic aerosol used was the same in both instances, now the retention pattern is completely reversed. Airways now contain more radioactive particles than the "cooler" parenchyma. Comparisons to photographs of the same lung (similar to Figure 1) clearly show that the red-orange-white areas correspond to airways.

The computer attached to the gamma camera translates the amount of activity in a region to color. The colors get warmer (red, orange, orange, white) as we get more and more activity. It is blue or black if there is very little or no activity. The same patterns of retained particles can be demonstrated with autoradiographs or by counting and weighing individual pieces from the lung slices (Valberg et al., 1982). These changes in retention pattern caused by breathing pattern will, over time, have a profound effect on the dose. Material deposited in airways will be cleared more quickly than particles deposited in the parenchyma.

CLEARANCE

It is essential to realize that different rates of clearance among species can also influence retention and, therefore, the total dose to the lung. All aspects of clearance may differ. We have recently examined mouse, hamster, rat, and rabbit in regard to the rate at which insoluble gold particles are taken up by pulmonary macrophages in situ (J. Brain, personal observation). Hamsters had the shortest half-time, followed by rats, rabbits, and mice. The observed in vivo differences were not completely accounted for by different in vitro activities of the macrophages or by different sizes or numbers of macrophages in the respiratory tract.

When the particles ingested by pulmonary macrophages are viable organisms, the efficiency with which the organisms are inactivated becomes a crucial factor in lung defense. The ability of the pathogen to cause lung damage and disease is in direct competition with the host's ability to mount an effective inactivation process. Different species demonstrate varying degrees of efficiency in this defense mechanism. When pulmonary antimicrobial defenses are examined, they also show marked species differences in the bactericidal activity of alveolar macrophages. For example, rabbit alveolar macrophages avidly ingest and kill Staphylococcus aureus, whereas alveolar macrophages from rats ingest but are rather poor at killing the same organisms. Also, Nguyen et al. (1982) reported significant differences in phagocytosis and killing by pulmonary macrophages from humans, rabbits, rats, and hamsters. In the absence of serum opsonins, macrophages recovered from humans were able to phagocytize Staphylococcus aureus, Cowan I (protein A positive). In contrast, pulmonary macrophages lavaged from rabbit, rat, and hamster, did not phagocytize Staphylococcus aureus, Cowan I, or other nonopsonized bacteria in the test system studied.

Felicetti and associates (1981) have shown that tracheal mucous velocity varies with species and is best correlated with tracheal surface area $^{0.57}$. They found that the clearance of intratracheally instilled ^{99}MTc-macroaggregates of albumin was found to be faster and more efficient in dogs than in smaller animals. If one includes non-mammalian species, even greater variability can be encountered. In boa constrictors, Grant et al. (1981) demonstrated that 41% of an inhaled radiolabeled submicrometric aerosol retained 5 hours after the end of the exposure was found in the trachea. This suggests a much slower mucociliary transport system than has been observed in mammals. Interestingly, Grant et al. (1981) also reported that less than 10% of the snake tracheal epithelium was ciliated. While studying chickens, Mensah and Brain (In press) showed that lung clearance of submicrometric particles has a biphasic pattern: a fast

phase, where clearance is faster than has been observed for hamsters or mice; and a slower phase. Also, Thomas (1972) has developed a model describing the kinetics of clearance of inhaled particles in the respiratory tract of mice, rats, and dogs.

Together, the relative rates of deposition and clearance determine the amount of a substance present in the respiratory tract; this is called the retention. If exposure is continuous, then the equilibrium concentration (achieved when the clearance rate matches the deposition rate), is also the retention. It is the retention integrated over time as well as the metabolism and properties of the particles that are presumably related to the magnitude of the toxic reponse.

In conclusion, it is important to remember that the species selected for exposure will influence the resulting dose to the lungs. Different species breathing the same aerosol do not receive identical doses. Exposure concentration (e.g., "mg/m^3" or "ppm") is not an adequate description of lung dose. Even when the same atmosphere is breathed by different species, very different amounts and distribution of retained particles may still result. There are both systematic and unusual variations in ventilation, collection efficiency, lung anatomy, and clearance mechanisms among species, which influence the local doses of retained aerosols and gases.

BIOLOGICAL RESPONSE

Even if different species had the same lung dose of a toxic particle or chemical, it is still unlikely that the extent of lung damage would be identical. Besides variations in deposition and clearance, varying responses also reflect differences in the activation, degradation, excretion, or mechanism of action of the compound in each species. The innate responsiveness of the analogous cell, tissue, or organ may also vary among species.

An example is the case of the anti-tuberculosis drug Isoniazid which is well tolerated at doses of 100 mg/kg in monkeys, whereas in dogs, doses of 20 mg/kg produce convulsion, respiratory failure, and death. It has been found that monkeys almost completely acetylate (thus inactivate) INH, while dogs cannot (Coulston, 1966).

The toxic effect of prolonged exposure to 100% O_2 has been known for a long time (Bean, 1945). Lung damage includes interstitial and alveolar edema, and progressive respiratory distress, sometimes leading to death (Crapo and Tierney, 1974). Among the mechanisms proposed for lung toxicity are the formation of free radicals, chain reactions, and destructive oxidations (Gerschman, 1964). One important free radical is the superoxide anion; the

development of tolerance to oxygen toxicity may involve the ability to dismutate the anion via an increase in the activity of pulmonary superoxide dismutase (SOD). Crapo and Tierney (1974) have shown that the rate of development of O_2 tolerance closely parallels the time-course for the increase in pulmonary SOD activity in the rat. However, in guinea pigs, hamsters, and mice who do not develop tolerance under similar circumstances as the rat, there was not as large an increase in SOD activity.

Frequently, different responses to toxic agents reflect anatomical differences. For example, there are significant interspecies differences in the fine structure of the respiratory tract which can result in differences in the degree and distribution of damage after exposure to harmful aerosols or gases. Several studies have shown that rats develop lesions in terminal bronchioles and alveoli of proximal alveolar ducts following exposure to ozone (Castleman et al., 1973), whereas in monkeys, the major focus of damage is the respiratory bronchiole (Dungworth et al., 1975). The differences in the distribution of damage may be explained in part by the structure of the respiratory tract epithelia. Respiratory bronchioles are well developed in Macaque monkeys and are lined by non-ciliated epithelium comprised of cuboidal cells interspersed among squamous cells (Castleman et al., 1979). Non-respiratory bronchioles are greatly abbreviated. Rats, on the other hand, do not have well-developed respiratory bronchioles; the pulmonary acinus is simpler, and the terminal bronchioles open into alveolar ducts.

A similar structural difference has been proposed as the basis for the resistance of avian lungs to inhaled O_2 at 1 ATA. When chickens and rabbits are exposed to 100% O_2, the rabbits die within 5 days, while the chickens remain unaffected at 19 days (Somayajulu et al., 1978). Oxidant-sensitive ciliated cells are found in the respiratory airways of rabbits but not in chickens, and this difference may well underlie the difference in response to hyperoxia observed in the two species (Somayajulu et al., 1978).

Different species breathing the same aerosol do not receive identical doses. Exposure concentration (e.g., "mg/m^3" or "ppm") is not an adequate description of lung dose. Even when the same atmosphere is breathed by different species, very different lung doses may still result. There are both systematic and unusual variations in ventilation, collection efficiency, lung anatomy, and clearance mechanisms among species which influence the amount and distribution of retained aerosols and gases. Many other differences in reponses exist among species, but all too frequently we lack even hypotheses for their existence. A comprehensive view of species differences, with predictive power, is lacking but remains a worthy goal.

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