

Pulmonary Distribution of Particles Given by Intratracheal Instillation or by Aerosol Inhalation

JOSEPH D. BRAIN, DWYN E. KNUDSON,¹ SERGEI P. SOROKIN, AND
MICHAEL A. DAVIS²

*Department of Physiology, Harvard University School of Public Health, Boston,
Massachusetts 02115*

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In animal studies concerned with the deposition of particulate matter in the lung, two methods for delivery of particles are commonly used, aerosol inhalation and intratracheal instillation of particle suspensions. We have attempted to evaluate the distribution patterns of each of these methods. Particles labeled with ^{99m}Tc were administered to both rats and hamsters. The animals were subsequently killed. The lungs were excised, weighed, inflated, dried, and divided into 54 pieces which were counted individually in a Nuclear-Chicago Model 4230 Automatic Gamma Scintillation System. Groups receiving intratracheal instillations demonstrated nonuniform distribution patterns with preferential deposition in the dependent portions of the lung. The aerosol groups evidence more even distribution with preferential deposition in the apical lobes.

INTRODUCTION

Toxic materials in the external environment can cross one of the body surfaces and penetrate to living cells; the most common pathway is the respiratory tract. Each day the average man breathes 20,000 liters of air laden with particles; many are deposited on the alveolar surface of approximately 70 m². In the laboratory, efforts are made to simulate this human exposure to particulate matter in animals. In other instances, the goal is to deliver to animals a disease-producing dose of air pollutants, carcinogens, radioactive particles, or other toxic particles, even though there may be no known human counterpart. In each instance, particles are usually introduced into the respiratory tract of experimental animals through two basic methods: In the first method, inhalation, the animals breathe an aerosol. In the second method, intratracheal instillation, a suspension of the particles in a carrier liquid is injected directly into the lumen of the trachea. Gravity causes the fluid and particles to run down into the dependent areas of the lung. The carrier liquid is rapidly absorbed into the pulmonary circulation, leaving the particles on the internal surfaces of the lung.

Each method has its merits and disadvantages. Although intuitively the inhalation technique seems to be more physiological, there are a number of advantages to using intratracheal instillations. Some of these are: (1) The actual dose delivered to each animal can be measured more accurately and administered more uniformly

¹ Current address: Section of Pulmonary Diseases, Department of Internal Medicine, College of Medicine, University of Arizona, Tucson, Arizona 85724.

² Current address: Department of Radiology, Harvard Medical School, 50 Binney Street, Boston, Massachusetts 02115.

with the intratracheal instillation technique. In inhalation exposures, the amount deposited is equal to the product of the minute ventilation times the collection efficiency of the animal. The collection efficiency, in turn, depends on the effective aerodynamic diameter of the aerosol, the breathing pattern of the animal, and the dimensions of the airways and alveoli. In intratracheal instillations, however, the same measured amount of material can be introduced into each animal. (2) The procedure is simpler (see Methods) and minimizes hazards to laboratory personnel when the materials are highly toxic (i.e., carcinogenic or radioactive). For example, experimental lung cancer has been studied in recent years by administering chemical or radioactive carcinogens directly into the trachea (Pylev, 1962; Shabad, 1962; Saffiotti *et al.*, 1968; Kennedy and Little, 1974). Aerosol generation is a complex, sophisticated art involving considerable technical skill (Mercer, 1973) while the technique of intratracheal instillation is much easier to master. When highly toxic materials are presented as aerosols, adequate safety precautions are more elaborate and difficult. Also, the cost of setting up aerosol generation systems and proper exposure chambers may be prohibitive compared to the negligible expense of giving intratracheal instillations. (3) The intratracheal instillation technique permits the introduction of large and therefore effective doses of material in a short time. For example, we exposed rats to 10 mg of coal dust by intratracheal instillation (Brain, 1971). To introduce this same quantity of material by inhalation requires a high aerosol concentration and a very long exposure period. (4) The intratracheal instillation techniques eliminates exposure to the skin or pelt. Aerosol exposures, unless they are head-only exposures, can involve large amounts of skin deposition. For some lipid-soluble materials, there may be percutaneous absorption; normal grooming habits may also result in the ingestion of significant amounts of particles. (5) Highly local exposures are possible. With the intratracheal instillation technique, monolateral or unilobular instillations may be carried out. This is a useful approach when it may be desirable to attempt to use the other parts of the lung as controls; in this way interanimal variability may be avoided. (6) This technique also allows the introduction of particle sizes which are normally nonrespirable. In some instances it may be difficult to obtain toxic or radioactive particles which are small enough to be deposited by inhalation in rodent alveoli. With intratracheal instillation techniques, even very large particles (20 to 100 μm) can easily be introduced into the alveolar region. This problem is accentuated by the fact the most small laboratory animals are obligatory nose-breathers, and thus considerable amounts of inhaled particles may be deposited in the nose and head and rapidly cleared to the gastrointestinal tract (Watson *et al.*, 1969). A related advantage is that with intratracheal instillations the site of deposition is relatively independent of particle size. During inhalation, however, inhaled aerosols of different sizes have different sites of deposition.

The primary objection to intratracheal instillations relates to the high probability that patterns of particle distribution in the lungs may be uneven and unlike those resulting from inhalation. The purpose of the experiments described here was to develop a method for examining the distribution of particles in rodent lungs, and to then compare the distribution of particles resulting from inhalation exposures with that from intratracheal instillations.

MATERIALS AND METHODS

Animals

Eighteen rats and 32 hamsters were exposed and analyzed. The Syrian golden hamsters were bred by Dennen Animal Industries (Gloucester, Massachusetts) and the Sprague-Dawley CD rats were supplied by Charles River Breeding Laboratories (Wilmington, Massachusetts). They were housed in individual wire cages and fed Purina Rat Chow and water *ad libitum*.

Preparation of Radioactivity Tagged Materials

Technetium-sulfur colloid (^{99m}Tc₂S₇) This agent was first introduced by Harper and coworkers (1965). It is currently used in nuclear medicine as a diagnostic imaging agent for liver, spleen, and bone marrow. The radiocolloid can be prepared by a variety of simple methods (Harper, 1965; Patton, 1966) or purchased in "kit" form from several commercial sources.

The particle size of the colloid used in this study (Tesuloid, E. R. Squibb and Sons) ranges from 0.01 to 3.0 μm (diameter) with 10% of the activity on particles less than 0.1 μm , 70% between 0.1 and 0.4 μm , 16% between 0.4 and 1.0 μm , and only 4% as particles exceeding 1.0 μm . The amount of free pertechnetate, that is technetium not bound to the sulfur colloid but present as the original pertechnetate (^{99m}TcO₄⁻), varied from 2 to 4%. The colloid is stable *in vitro* for at least 8 hours.

Technetium-iron hydroxide macroaggregates (^{99m}Tc-FHMA) This material was recently introduced as a diagnostic lung scanning agent (Yano *et al.*, 1969). It has been modified by one of us (Davis, 1971) to give a more satisfactory and reproducible product. The particle size of the macroaggregates ranges from 8 to 100 μm , with 36% of the activity on particles between 8 and 60 μm , 35% between 60 and 80 μm , and 29% between 80 and 100 μm . The macroaggregates contain 96 to 98% of the radioactivity present in the solution (2 to 4% unbound ^{99m}TcO₄⁻) and the increase in free TcO₄⁻ is only 2% 10 hours after preparation (0.2%/hour).

Aerosol Generation

Figure 1 shows a diagram of the aerosol generation and delivery system. The dispersion apparatus was similar to that used by Reist and Burgess to atomize polystyrene latex particles (1967). Air entered through a PEN-I-SOL nebulizer at a rate of 2.1 liters per minute. The aerosol was then combined with dilution air (3.3 liters per minute). This mixture passed through a drying tube 60.9 cm long and 7.4 cm in diameter. The residence time in the tube was sufficient to allow all the water to evaporate from the primary droplets, leaving only dry particles in the aerosol cloud. These particles, aggregates of the submicronic technetium colloid, were collected with an electrostatic precipitator and sized by electron microscopy (Phillips 100). The calculated mass median diameter was 0.87 μm , and the geometric standard deviation (σ_g) was 1.79.

The exposure chamber was a Plexiglass cube 25 cm on each edge. The aerosol was admitted to the chamber through a baffled inlet at the top, and left through a port in the lower wall. Groups of two hamsters and two rats were exposed to the technetium aerosol simultaneously for 1 hour. The animals were unrestrained and able to move freely within the box; they were moderately active during the first quarter of the 1-hour exposure while investigating their new environment, then

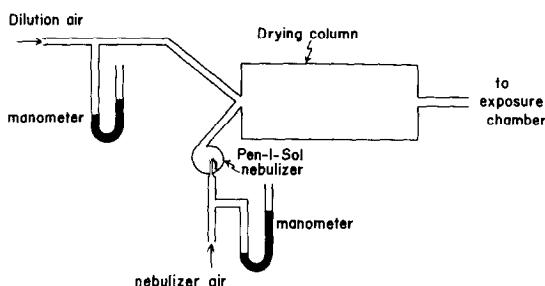


FIG. 1. The aerosol generation and delivery system.

their activity gradually decreased. The animals were killed by exsanguination within 5 minutes of the end of the aerosol exposure, and the lungs quickly excised.

In the studies designed to describe particle distribution morphologically, an iron oxide (Fe_2O_3) aerosol was used. Submicronic particles were produced from the combustion of iron pentacarbonyl, $\text{Fe}(\text{CO})_5$, in a furnace heated to 500°C. The result is a heterogeneous agglomerate aerosol which penetrates deep into the lungs where it is easily visualized by light and electron microscopy. Three hamsters were exposed for 3 hours to an aerosol of mass concentration, 300 mg/m³. Electron microscopy (Phillips 100) on a sample collected by electrostatic precipitation revealed a log normal size distribution with a count median diameter of 0.4 μm and a geometric standard deviation (σ_g) of 2.0. A more complete description of the aerosol and its generation has recently been published (Brain *et al.*, 1974).

Intratracheal Instillations

The hamsters were anesthetized with intraperitoneal injections of 0.6 cc of a 1% solution of sodium methohexitol (Brevital, Eli Lily & Co., Indianapolis, Indiana). Generally, the anesthetic effects of this rapidly metabolized barbiturate persisted for 5 to 10 minutes. The rats were anesthetized with ether. Once anesthetized, each animal was placed on a slanted board (20° from the vertical). As shown in Figure 2, the animal was supported by an elastic band under its upper incisors, and its mouth held open by a wire band across its lower incisors. A microscope lamp, with its beam directed at the neck area, provided transillumination. By opening the mouth of the animal and depressing the tongue, the larynx could easily be visualized.

The particle suspensions were delivered to the lungs through the trachea with a modified³ #19 gauge 3-in. B-D Yale Luer-Lok Spinal (Quincke)⁴ needle inserted between the vocal folds. The presence of the syringe needle in the trachea was verified at each instillation by gentle movement of the needle within the tracheal lumen in order to feel the cartilaginous rings. All animals received 0.15 ml suspensions per 100 g of body weight; the activity of the technetium suspensions was approximately 0.4 mc/ml. Except for the animals that were deliberately inverted immediately after the instillation, all animals were kept upright on the slanted board for 1 minute. The animals were then placed on their backs in their cages and were killed within 3 minutes.

³ The needle was bent to form a 135° angle and blunted on the end.

⁴ Becton, Dickinson & Company, Rutherford, New Jersey. Catalog No. 1141, 462LNR.

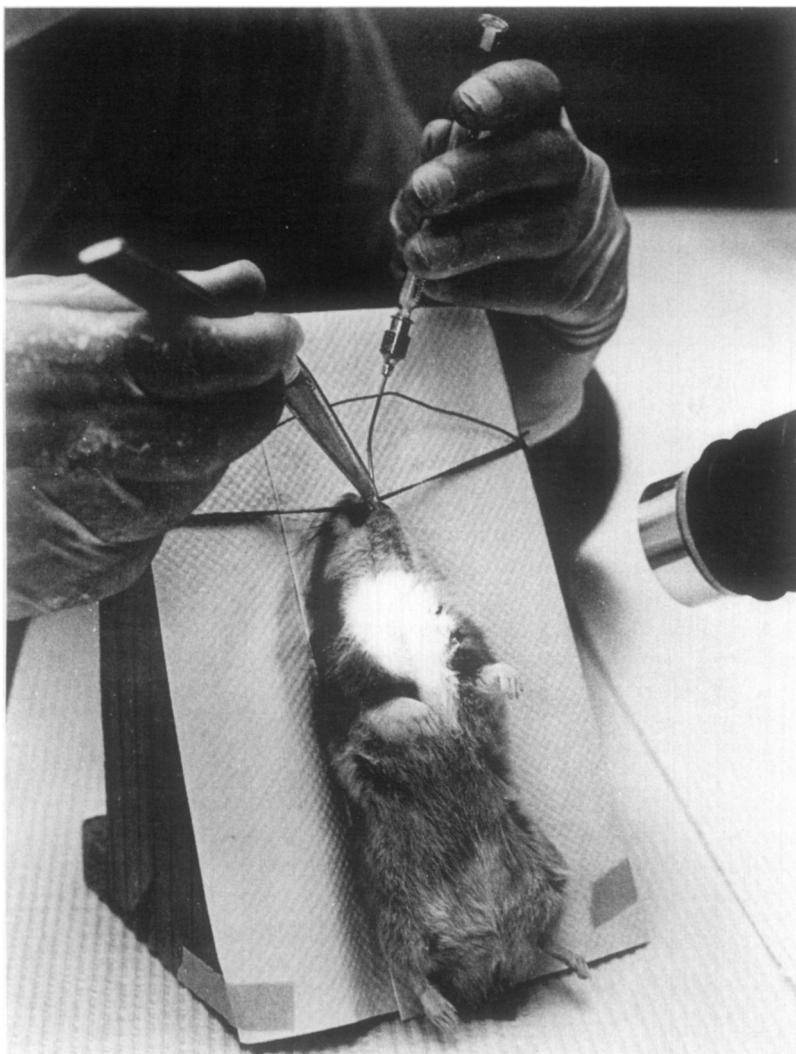


FIG. 2. Intratracheal instillation of hamster.

For morphological studies of distribution, three hamsters were given intratracheal instillations of a 1% suspension of submicronic iron oxide particles (Pfizer-#R-2999) in saline. As before, each animal received 0.15 ml/100 g of body weight.

Preparation and Analysis of Lungs

Following sodium pentobarbital (Nembutal, Eli Lily & Co., Indianapolis, Indiana) anesthesia, the animals were exsanguinated by cutting the abdominal aorta. The trachea was exposed and ligated. The thorax was opened by cutting up through the diaphragm and ribs, and the lungs and trachea were excised. The lungs were weighed, cannulated, and allowed to dry by being inflated with air and maintained at

a pressure of 30 cm H₂O (transpulmonary pressure) overnight. Small pieces of waxed paper inserted between the lobes during the drying process prevented sticking, so that the individual dried lobes could be separated easily.

We attempted to dissect all lungs in a predetermined and reproducible fashion as shown in Fig. 3A and B. Each lung and lobe was separated by cutting the extrapulmonary bronchi close to the parenchyma. The position of the lungs in the intact animal were estimated by X-ray (70 Kvp, Picker X-Ray Corporation, Style 1344C). Whole body films were taken in the intratracheal position and in positions characteristic of unanesthetized animals.

Slices were made in a plane approximately perpendicular to the axis of each entering lobar bronchus. The unilobular left lung was sliced into six slices of approximately equal thickness. Each slice was then subdivided into two to four pieces (now shown in Fig. 3). The right lung was separated into its four component lobes: the upper, the middle, the cardiac, and the lower lobe. These lobes were also sliced in a consistent manner and the slices cut into pieces. To minimize the possibility of cross-contamination, clean razor blades and a fresh surface were used for each cut. Thus, for each animal, the lungs were divided into 23 slices, which were in turn divided into a total of 54 specific pieces. The pieces from each animal were placed individually into plastic counting tubes, capped, and counted in a Nuclear-Chicago Model 4230 Automatic Gamma Scintillation System. Samples of the original technetium suspension were counted in series with the lung samples, and were used to correct for decay. After counting the lung pieces were placed in an 110°C oven until thoroughly dry and then weighed.

Calculations

A Fortran program (IBM System 360) was written to compile and evaluate the data obtained. Data were entered on punched cards. The program subtracted

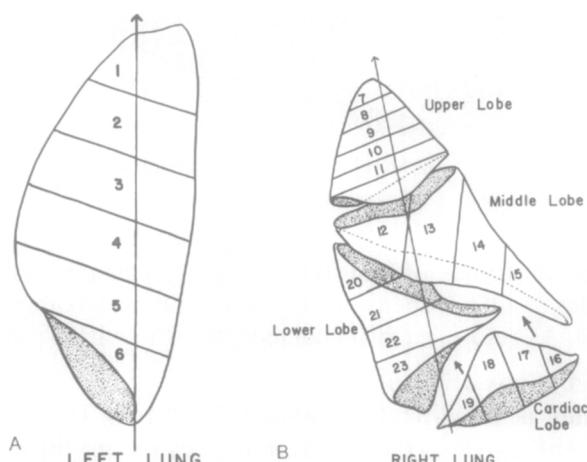


FIG. 3. A. Diagram of left lung showing location of slices 1 through 6. The arrow indicates the vertical direction for intratracheal instillations. B. Diagram of the right lung showing the four lobes and the location of slices 7 through 23. As before, the arrow indicates the vertical direction.

background, corrected the counts for decay and calculated the counts per minute per milligram of dry lung for each piece. This was compared to the activity of the lung as a whole and an evenness index (EI) was then calculated for each piece. The ratio of total counts to total weight for both lungs was compared to the ratio of the counts of each piece to its weight. Thus,

$$\text{EI.} = \frac{\text{counts per minute per gram lung}_{\text{piece}}}{\text{counts per minute per gram lung}_{\text{whole lung}}} \times 100$$

An EI below 100% indicates that the piece in question received less than its share of radioactivity; similarly a piece with an EI greater than 100% received more than its share of radioactivity. In an idealized lung with an absolutely even distribution pattern, all pieces should have an EI of 100%. The degree of departure from 100% indicates how unevenly the particles are distributed. The EI was also calculated for the slices and lobes of each lung. The mean, standard deviation and standard error were then found for each numbered piece, slice, lobe, and lung for the animals in each experimental group. Paired groups of animals were compared by applying Students' *t*-test to the data and by calculating the corresponding *P*-value.

Histological Procedures

Excised lungs were fixed at pH 7.2 to 7.3 by immersion in a glutaraldehyde-formaldehyde mixture containing 0.01% trinitroscresol (Ito and Karnovsky, 1968). Small pieces were embedded in glycol methacrylate, and 1 μm plastic sections were prepared. Although ferric oxide particles are visible by virtue of their brick red color, additional contrast may be given the particles if sections are stained for iron by the Prussian blue reaction. In Perl's method, sodium ferrocyanide is reacted with ferric ions in a dilute solution of hydrochloric acid to produce ferric ferrocyanide. The sections were given a reddish counterstain by 10 to 30 minutes' exposure to a 1% aqueous solution of basic fuchsin. Additional details pertinent to histological procedures for examining iron compounds were recently published (Sorokin and Brain, 1975).

Experimental Design

In order to quantify the distribution patterns, 50 animals, divided into eight groups, were analyzed as shown in Table 1. The first four groups consist of rats and hamsters exposed to technetium—sulfur colloid, either by aerosol or by intratracheal instillation. In order to investigate whether the nonuniformity of distribution seen in intratracheally instilled animals was random or systematic, a fifth group of hamsters was given a series of five intratracheal instillations. They were given at 2-hour intervals, each accompanied by Brevital anesthesia; the animals were killed immediately after the fifth instillation. In the sixth group, hamsters were given a single instillation, were immediately inverted and held in the head-down position for a period of 1 minute just prior to sacrifice. In groups seven and eight, rats and hamsters received intratracheal instillations of technetium iron hydroxide macroaggregate. The macroaggregate has a mass median diameter of about 70 μm whereas the sulfur colloid is submicronic in size. These last two groups were included to investigate the effect of particle size on the distribution of intratracheally instilled particles.

TABLE 1
EXPERIMENTAL DESIGN

Group number	Species	Exposure method	Particle used	Remarks	Number of animals
1	Rat	Inhalation	Tc-S-Colloid	—	6
2	Rat	Intratracheal	Tc-S-Colloid	—	6
3	Hamster	Inhalation	Tc-S-Colloid	—	6
4	Hamster	Intratracheal	Tc-S-Colloid	—	6
5	Hamster	Intratracheal	Tc-S-Colloid	Multiple instillations	8
6	Hamster	Intratracheal	Tc-S-Colloid	Inverted	6
7	Hamster	Intratracheal	Tc-Fe(OH) ₂ Macroaggregates	—	6
8	Rat	Intratracheal	Tc-Fe(OH) ₂ Macroaggregates	—	6
				Total	50
9	Hamster	Inhalation	Fe ₂ O ₃	Morphological assessment	3
10	Hamster	Intratracheal	Fe ₂ O ₃	Morphological assessment	3
				Total	6
				Grand total	56

For each group of animals, the mean EI, the standard deviation (SD) and standard error (SE) were calculated for each of the 54 pieces. The EI, SD, and SE were also calculated for each of the 23 slices and each of the four lobes of the right lung, as well as the left lung *in toto*. All these data are available upon request from the authors. The most interesting and provocative data will be presented in Results and Discussion.

In order to provide greater microscopic detail and illustrate qualitatively the differences in distribution patterns between inhalation and intratracheal exposures, six additional hamsters were administered ferric oxide particles as shown in Table 1 and as described earlier in the Methods section. All animals were killed by exsanguination within 1 hour of the intratracheal instillation or the termination of the aerosol exposure.

RESULTS AND DISCUSSION

Distribution Patterns

Table 2 shows data obtained from hamsters exposed to aerosols and to intratracheal instillations. The main body of the table lists the average EIs (\pm SE) for all slices from groups 3 and 4.⁵ The column entitled "Probability" indicates the likelihood that the observed difference between the two groups would have occurred by chance as predicted by Students' *t*-test. *P*-values shown with an asterisk are less than 0.05 and assumed to be significant. There are many areas of the lung where significant differences occur. For example, in slice number 1 of the left lung, the most apical slice, the aerosol-exposed animal has an EI of 129%, while the intratracheally instilled animal has an EI of only 21%. The entire right upper lobe is dramatically different between the two groups; all slices from the aerosol-exposed animals have EIs which are significantly greater than 100%. The intratracheally instilled animals, however, have EIs ranging from 2 to 51%. It can be seen that the

⁵ Tables 2 and 3 give only the data for slices, lobes, and lungs. Similar data for the 54 pieces are available on request from Dr. Brain.

right middle and right lower lobes also differ significantly between the two groups of animals. Similar results were obtained for groups 1 and 2 (rats) as summarized in Table 3. The data suggest that deposition patterns are markedly different between the animals exposed by inhalation and those exposed by intratracheal instillation.

What is the nature of the deposition patterns for each of the two exposure techniques? In the animals breathing the aerosol, there is generally more deposition in the apical parts of the lung and less in the basilar regions. This can be seen in the left lung in hamsters, for example, where the EI gradually falls from 129% at the

TABLE 2
HAMSTER LUNGS

Location	Slice number	Group 3	Group 4	Probability ^a
		Aerosol inhalation	Intratracheal instillation	
Left lung	1	129 ± 15%	21 ± 17%	<.001*
	2	101 ± 11	67 ± 24	.230
	3	112 ± 08	97 ± 16	.391
	4	103 ± 09	116 ± 19	.527
	5	87 ± 04	148 ± 45	.206
	6	60 ± 05	126 ± 33	.074
Total left lung		98 ± 05	107 ± 16	.633
Right upper	7	129 ± 28%	02 ± 01%	<.001*
	8	148 ± 12	26 ± 13	<.001*
	9	126 ± 11	48 ± 09	<.001*
	10	144 ± 08	51 ± 16	<.001*
	11	185 ± 30	33 ± 20	.002*
Total lobe		142 ± 05	39 ± 10	<.001*
Right middle	12	134 ± 16%	05 ± 03%	<.001*
	13	114 ± 07	15 ± 05	<.001*
	14	106 ± 13	39 ± 09	.002*
	15	94 ± 12	57 ± 14	.075
Total lobe		105 ± 08	38 ± 08	<.001*
Right cardiac	16	62 ± 08%	48 ± 41%	.745
	17	117 ± 31	68 ± 25	.243
	18	97 ± 10	131 ± 22	.186
	19	90 ± 17	110 ± 18	.444
Total lobe		95 ± 09	99 ± 18	.851
Right lower	20	93 ± 04%	97 ± 15%	.841
	21	102 ± 05	194 ± 49	.089
	22	86 ± 07	124 ± 23	.148
	23	61 ± 12	95 ± 40	.445
Total lobe		91 ± 05	135 ± 17	.029*
Total right lung		102 ± 08	97 ± 21	.830

^a Indicates probability that the observed difference between means of groups 3 and 4 occurred by chance (Students' *t*-test).

* Probability less than 1 in 20 ($P < .05$).

TABLE 3
RAT LUNGS

Location	Slice number	Group 1	Group 2	Probability ^a
		Aerosol inhalation	Intratracheal instillation	
Left lung	1	143 ± 06%	24 ± 10%	<.001*
	2	111 ± 07	88 ± 16	.234
	3	92 ± 03	107 ± 19	.461
	4	105 ± 02	135 ± 16	.100
	5	96 ± 04	127 ± 16	.087
	6	79 ± 04	82 ± 21	.891
Total left lung		100 ± 01	112 ± 07	.106
Right upper	7	149 ± 09%	29 ± 11%	<.001*
	8	167 ± 17	75 ± 21	.007*
	9	153 ± 09	103 ± 23	.073
	10	148 ± 14	132 ± 27	.614
	11	139 ± 13	50 ± 17	.002*
Total lobe		151 ± 08	98 ± 19	.030*
Right middle	12	77 ± 06%	22 ± 09%	<.001*
	13	96 ± 07	35 ± 13	.002*
	14	98 ± 03	47 ± 18	.017*
	15	77 ± 04	52 ± 17	.192
Total lobe		89 ± 03	44 ± 15	.013*
Right cardiac	16	70 ± 05%	28 ± 11%	.006*
	17	92 ± 04	88 ± 22	.857
	18	94 ± 05	126 ± 35	.371
	19	88 ± 10	78 ± 26	.713
Total lobe		88 ± 02	90 ± 18	.909
Right lower	20	83 ± 05%	102 ± 07%	.046*
	21	105 ± 02	128 ± 20	.297
	22	93 ± 04	115 ± 25	.407
	23	72 ± 06	94 ± 15	.221
Total lobe		93 ± 02	112 ± 14	.196
Total right lung		100 ± 01	93 ± 09	.456

^a Indicates probability that the observed difference between means of groups 1 and 2 occurred by chance (Students' *t*-test).

* Indicates that the probability is less than 1 in 20 ($P < .05$).

apex of the left lung to 60% at the base. In the rats, the EI falls from 143% to 79% as one moves from the apex to the base of the left lung. If one examines the lobar data, one can see that in the hamsters the EI falls from 142% (right upper) to 91% (right lower). In the rat, the EI falls from 151% (right upper) to 93% (right lower).

The initial distribution pattern of deposited particles reflects the distribution of ventilation in rodent lungs as well as the local collection efficiency. This pattern may then be modified by clearance processes operating during the 1-hour exposure period and during the 5-minute interval between the exposure termination and

sacrifice. Although Agostoni and D'Angelo (1971) have made some measurements of pleural pressure gradients in small animals, we are aware of no information regarding the regional distribution of inspired air in hamsters and rats. Interpretation of our data is further complicated by our inadequate knowledge of the exact position of the lungs *in situ* during spontaneous breathing of the radioactive aerosol. Observations during 1-hour exposure period indicate that unrestrained hamsters and rats spend much of their time in standing and intermediate positions as well as the more frequent prone position, so the reader is cautioned that apical and basilar slices refer to anatomical positions and do not accurately describe the position of that slice in relation to gravity. Additional studies utilizing controlled postures are currently underway; these studies are designed to examine the deposition patterns in greater detail.

During the intratracheal instillations, since the animals are anesthetized and positioned on a slanted board, the geometry is better fixed. Figures 3 A and B indicate the vertical direction as judged by X-ray of the rats. Now the apical regions correspond to the uppermost portions in the gravitational as well as the anatomical sense. Animals receiving intratracheal instillations show opposite patterns from those exposed by aerosol; the apical portions of the lung receive less and the more dependent portions receive more. Predictably carrier fluids generally flow down-hill. For example, in Table 2 one can see that the EI increases as one goes from the top to the bottom of the left lung. In the lobar data, the right upper lobe has only 39% of its share, while the right lower lobe has 135% of its share. The rat data in Table 3 generally show similar distribution effects with apical slices having less activity. In some slices, however, there is evidence that some of the instilled fluid may have gone "uphill." Slice No. 1 in the left lung, for example, is clearly above the carina. Fluid may reach these areas by capillary action or by pressure differences developed across instilled fluid which may block small airways.

It is important to note that the animals were killed immediately after either the 1-hour exposure to the aerosol or the intratracheal instillations. Therefore, the analysis examines the distribution of particles deposited in airways as well as in lung parenchyma. It is likely that if sufficient time were given to allow complete clearance from the airways, the pattern of retained particles may have been altered.

Other Comparisons.

In Table 4 sample data from all eight groups are given.⁵ Although many comparisons can be made among these groups, differences will only be commented on briefly. The effects of species differences on distribution of particles were examined. When data from the rat and hamster aerosol exposures were compared, the differences were relatively small. Only three out of a possible 23 slices showed significant species differences in regard to distribution patterns, and these differences were relatively small. None of the lobar data showed significant differences between rats and hamsters. When intratracheal instillations in hamsters and rats were compared, again three slices out of 23 were significantly different at or below the 0.05 level. All three slices that were significantly different were found in the right upper lobe, and in the grouped lobar data there was a significant difference between the right upper lobes in rats and hamsters. The right upper lobe in the hamsters had an EI of $.39 \pm .11$ while the right upper lung in the rats had an EI of $.98$.

TABLE 4
SELECTED EVENNESS INDICES: ALL EIGHT GROUPS^a
ALL ENTRIES ARE MEAN \pm SE, AS A PERCENTAGE.

Location	Slice number	Tc-S-Colloid particle used						Rc-Fe(OH) ₂ macroaggregates used		
		Rat			Hamster			Rat		
		Inhalation	Instillation	Inhalation	Instillation	Instillation	Instillation	Group 5 ^b	Group 6 ^b	Group 7
Right upper	7	149 \pm 09	29 \pm 11	192 \pm 28	02 \pm 01	23 \pm 03	06 \pm 04	41 \pm 38	08 \pm 03	
	8	167 \pm 17	75 \pm 21	148 \pm 12	26 \pm 13	60 \pm 11	65 \pm 20	37 \pm 25	47 \pm 15	
	9	153 \pm 09	103 \pm 23	126 \pm 11	48 \pm 09	99 \pm 10	122 \pm 35	45 \pm 15	111 \pm 34	
	10	148 \pm 14	132 \pm 27	144 \pm 08	51 \pm 16	105 \pm 17	193 \pm 62	115 \pm 21	127 \pm 17	
	11	139 \pm 13	50 \pm 17	185 \pm 30	33 \pm 20	69 \pm 12	97 \pm 46	107 \pm 24	142 \pm 47	
Total lobe		151 \pm 08	98 \pm 19	142 \pm 05	39 \pm 11	80 \pm 10	106 \pm 80	71 \pm 17	93 \pm 20	
Right middle	12	77 \pm 06	22 \pm 09	134 \pm 16	05 \pm 03	12 \pm 03	03 \pm 01	38 \pm 24	12 \pm 04	
	13	96 \pm 07	35 \pm 13	114 \pm 07	15 \pm 05	35 \pm 07	48 \pm 20	83 \pm 15	38 \pm 13	
	14	98 \pm 03	47 \pm 18	106 \pm 13	39 \pm 09	75 \pm 10	85 \pm 24	135 \pm 14	58 \pm 18	
	15	77 \pm 04	52 \pm 17	94 \pm 12	57 \pm 14	92 \pm 06	72 \pm 21	110 \pm 18	55 \pm 14	
Total lobe		89 \pm 03	44 \pm 15	105 \pm 08	38 \pm 08	70 \pm 06	63 \pm 48	99 \pm 07	46 \pm 12	
Total right cardiac lobe		88 \pm 02	90 \pm 18	95 \pm 09	99 \pm 18	105 \pm 10	98 \pm 24	107 \pm 22	110 \pm 14	
Total right lower lobe		93 \pm 02	112 \pm 14	91 \pm 05	135 \pm 17	99 \pm 06	85 \pm 71	77 \pm 09	113 \pm 10	
Total right lung		100 \pm 01	93 \pm 09	102 \pm 08	97 \pm 21	95 \pm 03	84 \pm 25	86 \pm 13	96 \pm 17	
Total left lung		100 \pm 02	112 \pm 17	98 \pm 15	107 \pm 39	116 \pm 09	133 \pm 48	132 \pm 27	109 \pm 29	

^a Animals received five multiple instillations.

^b Animals received single instillation and were immediately inverted for a period of 1 minute.

$\pm .19$ (significant at the 0.02 level). These differences may represent different branching angles of the lobar bronchus supplying the right upper lobe in the two species.

The effect of inverting the hamster immediately following the intratracheal instillation appeared to be relatively small. A comparison of groups 4 (intratracheal upright) and 6 (inverted following intratracheal instillation) revealed that no pieces or lobes were significantly different, and only one out of 23 slices differed significantly ($p < 0.5$), an occurrence to be expected by chance. However, as can be seen in Table 4, the data suggest that increased amounts of particles reached the right upper lobe when the animal was inverted. In the inverted hamsters, slices 11 through 15 and the right upper lobe as a whole all have more than twice the activity of the corresponding regions in the hamsters which remained upright. The three most apical slices from the left lung show the same pattern. Similarly, the most basilar slices of the right upper and left lobes show a reduction of activity in the inverted animals. Collectively, these data suggest that the absorption of the carrier fluid is not instantaneous. Rather, the mobility of the fluid persists long enough to make possible some redistribution of the particles.

A comparison of the effect of particle size on distribution of intratracheally instilled particles (group 2 versus group 8, or group 4 versus group 7) suggests that changing size from submicronic colloids to $70\mu\text{m}$ macroaggregates has only a small effect on distribution, as can be seen in Table 4. Statistical analysis of the original data reveals that no rat slices or lobes are significantly different ($P < 0.05$) when the two different particle sizes are compared. In the hamsters, the intralobar patterns for the macroaggregates are also much like those produced by the colloid; the familiar pattern of increasing EIs from top to bottom clearly persists. However, six out of 26 slices (all in the right upper or middle lobes) did differ significantly. The macroaggregates deposited more in the right middle lobe and less in the right lower lobe than did the colloidal particles. In spite of this small but measurable effect, it does appear that the distribution of particles intratracheally instilled is primarily, but not exclusively, determined by the properties and volume of the carrier fluid, not by the properties of the particles. It is likely that if the density or rheologic properties of the carrier fluid were altered, the distribution of the intratracheally instilled particles would be altered as well. Distribution is also dependent upon the volume of fluid instilled. For example, if only 0.01 cc/100 g body weight were instilled, very little of the carrier fluid and particles would penetrate past the airways. On the other hand, as larger volumes of fluid are instilled, the fluid is able to run deeper into the lung. Baxter and Port (1974), using a specially prepared intratracheal speculum, instilled volumes as large as 1.5 ml into hamster lungs and reported increased penetration to the periphery. All the experiments reported here deal with one volume, 0.15 cc/100 g body weight. We selected this volume since it is close to volumes instilled by many other investigators (Saffioti *et al.*, 1968; Brain, 1971; Kennedy and Little, 1971).

Uniformity of Distribution

A major purpose of these experiments was to describe the uniformity of the distribution of the particles as they usually are administered by these two common techniques (i.e., instilled while anesthetized and upright, and inhaled while awake

and unrestrained). We estimated the uniformity of distribution in two ways. (1) by calculating the coefficient of variation for the average data, and (2) by examining the distribution of the EIs of the individual pieces.

Coefficients of Variation

Table 5 shows the average coefficient of variation for data from the eight experimental groups. The coefficient of variation is calculated by dividing the standard deviation by the mean; the greater the variability of the data, the greater the coefficient of variation. Values for all slices or lobes from each group were averaged to produce the mean coefficients shown in Table 5. It can be seen that the aerosol-exposed animals (groups 1 and 3) always have smaller coefficients of variation than do the intratracheally instilled animals. Also of considerable interest is the diminished coefficient of variation caused by multiple intratracheal instillations.

Distribution of EIs

Another way of examining the uniformity of distribution is to look at the range of EIs for each group of animals. In Table 6 the distribution of EIs for the eight groups can be seen. Evenness indices ranged from a low approaching 0 to 400%. This total range was divided into twenty groups and the data arranged as shown. If the activity were ideally distributed all pieces would lie near 100%. In this table, each piece from each of the six animals is scored individually. The total at the bottom represents the number of animals in each group times 54. In groups 4 and 6, one piece was lost and is not included. It can be seen in groups 1 and 3, the aerosol-exposed animals, that very few pieces have EIs below 40%. On the other hand, in the intratracheally instilled groups, there are many pieces which have EIs below 40%. In addition, the number of pieces with EIs greater than 200% is considerably greater in the intratracheally instilled animals as compared with the aerosol exposed animals.

Selected data from Table 6 are graphically shown in Figure 4. The mode of the curve for the aerosol exposed animals lies around 1. The distribution is rather narrow with a slight tail to the right. The mode of the intratracheally instilled animals, however, lies in the 0 to 20% group, the curve is much less symmetric and is spread out to a greater degree.

In Figure 5, three curves based on the hamster data are shown. The aerosol inhalation and single intratracheal instillation show the same relationship as was shown in rats (Fig. 4). The additional curve shows the effect of five multiple

TABLE 5
AVERAGE COEFFICIENTS OF VARIATION

Group no.	Description	Slices	Lobes
1	Rat—Aerosol inhalation	14.3%	6.2%
2	Rat—Instillation, colloid	64.4	45.1
3	Hamster—Aerosol inhalation	26.6	16.0
4	Hamster—Instillation, colloid	85.2	45.7
5	Hamster—Multiple instillations, colloid	43.8	23.8
6	Hamster—Inverted	81.4	59.2
7	Hamster—Instillation, macroaggregates	84.7	35.2
8	Rat—Instillation, macroaggregates	60.1	39.1

TABLE 6
DISTRIBUTION OF EVENNESS INDICES

Evenness index (%)	Group								
	1	2	3	4	5	5**	6	7	8
0- 20	1	49	0	79	32	24.00	63	64	44
21- 40	2	31	8	36	43	32.25	51	45	39
41- 60	12	33	25	30	57	42.75	29	32	39
61- 80	50	32	50	39	61	45.75	32	20	35
81-100	84	35	71	25	58	43.50	26	25	43
101-120	82	40	66	18	71	53.25	21	20	21
121-140	44	33	42	20	42	31.50	15	28	21
141-160	20	30	23	18	30	22.50	12	30	19
161-180	11	17	20	25	14	10.50	23	15	13
181-200	11	9	6	11	9	6.75	9	14	13
201-220	3	4	2	7	10	7.50	10	3	15
221-240	3	3	4	3	2	1.50	9	7	4
241-260	0	2	2	3	1	0.75	6	4	3
261-280	1	3	0	2	1	0.75	3	1	3
281-300	0	3	1	3	1	0.75	5	6	5
301-320	0	0	2	2	0	0.00	2	4	3
321-340	0	0	0	0	0	0.00	4	1	2
341-360	0	0	1	2	0	0.00	4	2	0
361-380	0	0	1	1	0	0.00	0	2	1
381-400	0	0	0	0	0	0.00	0	1	1
Total*	324	324	324	323	432	324	323	324	324

* Most groups had six animals and therefore, 6×54 , or 324 pieces. In both groups 4 and 6, one piece was lost. Group 5 is composed of eight animals.

** Group 5 is composed of 8 animals, and thus there are more pieces in each interval. Column 5** shows the normalized values based on the assumption that only six animals had been examined, thus allowing comparison with other groups.

intratracheal instillations on the distribution of activity. We sought to determine to what degree the nonuniformity observed in the intratracheal instillations was systematic, and to what degree random. If the nonuniformity was entirely random, then multiple intratracheal instillations should reduce the nonuniformity. However, if some of the nonuniformity is systematic, then that nonuniformity should persist regardless of the number of intratracheal instillations.

It can be seen that some of the nonuniformity is random; in other words, it is considerably reduced by multiple instillations. The multiple intratracheal instillation curve has become more like the aerosol inhalation curve (its mode is now between 101 and 120%). However, there are still 75 pieces (17%) which have EIs less than 40% as compared to the aerosol inhalation where only eight pieces (2.5%) have an EI of less than 40%. We have assumed that previous instillations had a negligible effect on the fate of subsequent injections. It is possible that intratracheal instillations or the transient anesthesia may influence the caliber of airways, the quantity or quality of mucus in the airways, and hence, the direction and depth of the instilled fluid. A deliberate attempt was made to minimize these effects by

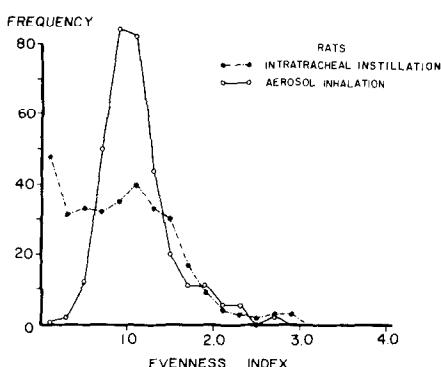


FIG. 4. Rat, Evenness Indices: aerosol inhalation versus intratracheal instillations.

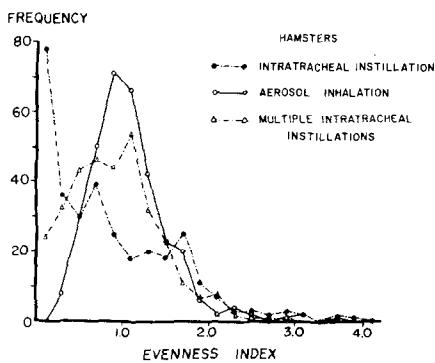


FIG. 5. Hamster, Evenness Indices: aerosol inhalation, intratracheal instillations, multiple intratracheal instillations.

waiting 2 hours between each instillation; this provided considerable time for the carrier fluid to be absorbed, and for the lung and airways to return to normal.

Morphological Assessment

The data just presented clearly indicate important differences between particle distribution patterns as established by inhalation, as contrasted with those obtained by intratracheal instillations. The following data show that these two distribution patterns are also readily distinguished when the lungs from experimental animals are removed shortly after exposure and given over to morphological study. Since the mass of technetium labeled particles given is very small and not readily visible, iron oxide particles (see Methods) were used in these experiments.

Major differences in distribution patterns are visible to the naked eye in whole amounts of the exposed lungs after they have been cleared and embedded in plastic. In intratracheally instilled specimens, the main airways, together with the most directly accessible alveoli, are rendered opaque by the rusty deposits to produce the pattern of a floriated bronchial tree centered in an otherwise clear lung. By way of contrast, in specimens recovered from inhalation exposures, airway outlines are only moderately enhanced by the particulate deposits; and, depending on conditions of exposure, the dust may impart a discernable coloration to the peripheral

lung as well; but the instillation pattern of a floriated bronchial tree is not seen.

Histologically, the appearance of the sectioned lung is consistent with the picture gained from examination of the whole mounts. Figures 6 through 9 present the contrasting appearances of hamster lungs 1 hour after cessation of the intratracheal and inhalation exposures; an instillation of 0.15 ml of a 1% suspension of submicronic iron oxide particles in saline, and a 3-hour exposure to an iron aerosol at a concentration of 300 mgm/m³, respectively. The localizations of iron oxide are taken to represent mainly the deposition patterns in these lungs, although, as discussed earlier, these patterns have been modified by clearance mechanisms.

At low magnification, the instilled particles are visible as heavy deposits on the surfaces of bronchi and the more proximal alveoli of the lung, such as those along the hamster's short respiratory bronchioles and the alveolar ducts. Collectively these deposits extend only part way towards the peripheral margins of the lung (Figure 6 broken line). At the same magnification, the iron oxide deposits in the lung exposed by inhalation are scarcely visible on respiratory surfaces, but are seen as they become concentrated in alveolar macrophages (Figure 7, open circles). Such particle-laden cells are widely encountered among pulmonary alveoli in this lung, including the alveoli adjacent to the pleura. Consequently, it is clear that use of the inhalation method, as compared with the intratracheal instillation procedure, has resulted in a deeper penetration of particles into the respiratory zone and a more uniform exposure of pulmonary tissues.

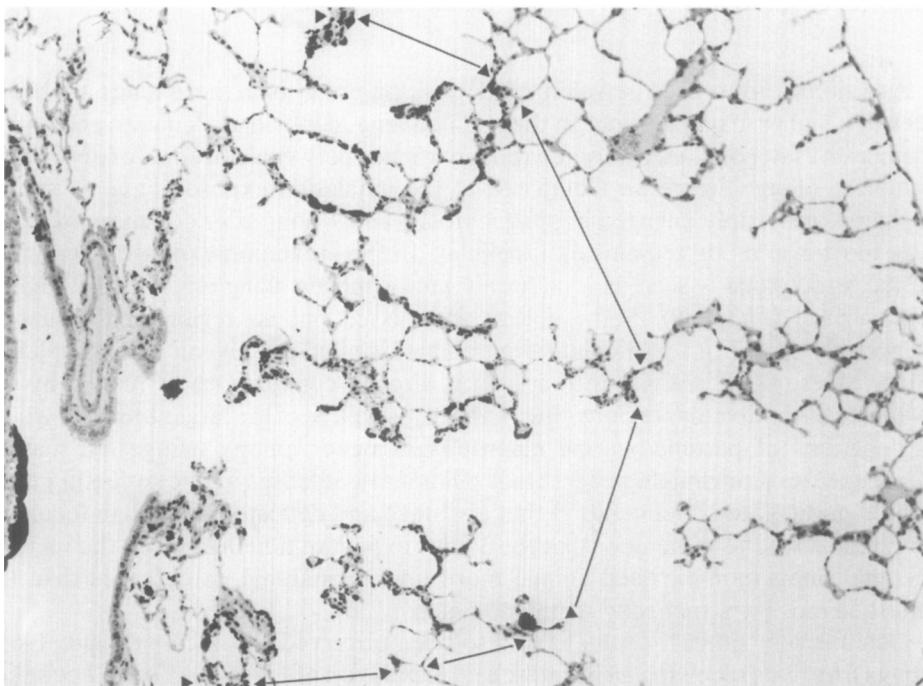


FIG. 6. Intratracheal instillation in hamster lung. Iron oxide (black) coarsely deposited on surfaces of bronchi and proximal alveoli, absent from periphery (beyond broken line). Prussian blue and basic fuchsin. $\times 125$.

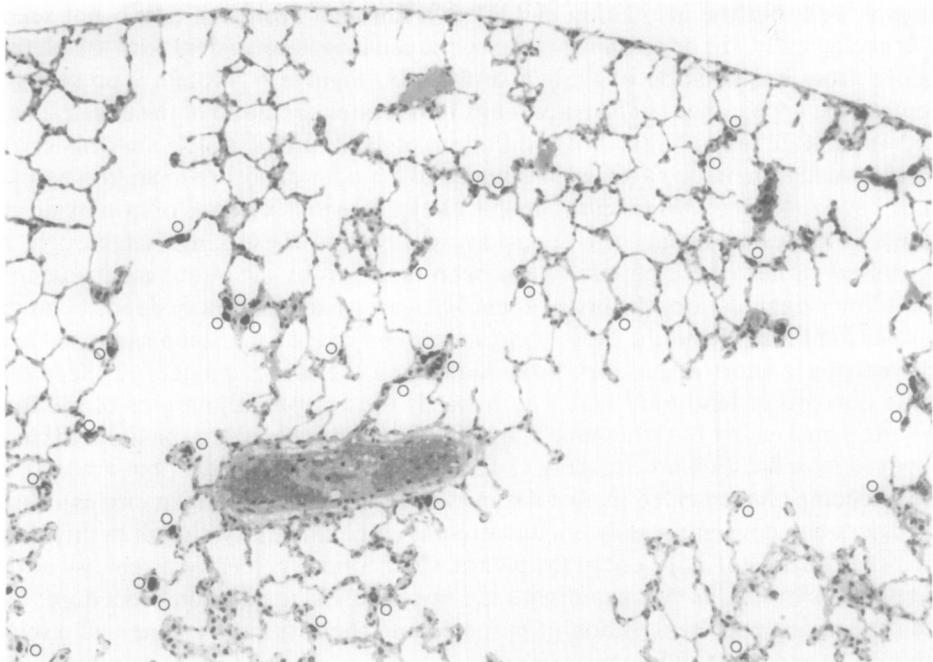


FIG. 7. Inhalation exposure of hamster lung. Iron oxide visible in alveolar macrophage (open circles) widely scattered over field of view. Prussian blue and basic fuchsin. $\times 125$.

Additional differences between the deposition patterns in these lungs are better seen at a higher magnification. In the instilled lung, deposits are coarse, and within the region of deposition, alveolar exposure ranges between extremes of absence to complete filling (Figure 8). Compared to the inhalation exposed lung, few macrophages are visibly engaged in phagocytosis, and 1 hour after cessation of exposure most iron oxide remains extracellular. In the inhalation-exposed lung, uningested ferric oxide is seen as a uniformly faint stippling along much of the respiratory surface (Figure 9). Some alveoli are missed but no region of the lung is completely free from particulate contamination, and no alveoli are completely filled. Macrophage activity in this lung is already conspicuous as judged by the evidence of the alveolar regions (Fig. 7 and 9), as well as of the larger bronchi, where aggregations of particle-bearing macrophages occur among uningested matter. While the foregoing might suggest that pulmonary defenses are better equipped to handle particulate contamination that is diffuse and delicate rather than focal and heavy, it is well to remember that the 3 hour exposure time had given the unanesthetized, inhalation-exposed animal more time to marshal its defenses than the anesthetized, intratracheally instilled animal.

Qualitatively similar results have also been observed in six cats (Brain, 1966). Lungs from cats receiving an intratracheal instillation of 1.5 mg carbon in 2 cc saline 3 hours before death, demonstrated strikingly uneven distribution of the instilled carbon particles. Most alveoli received no carbon. Other areas, particularly some of the small airways, had large quantities of carbon particles.

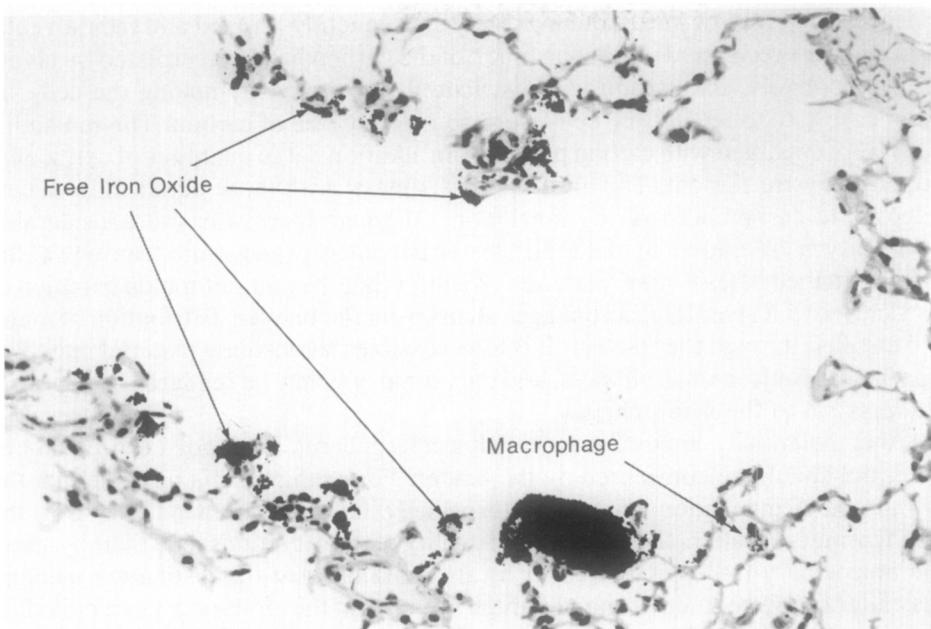


FIG. 8. Intratracheal instillation in hamster lung, 1 hour after exposure. Particle deposition along alveolar duct is heavy and uneven, few macrophages yet present. Prussian blue and basic fuchsin. $\times 250$.

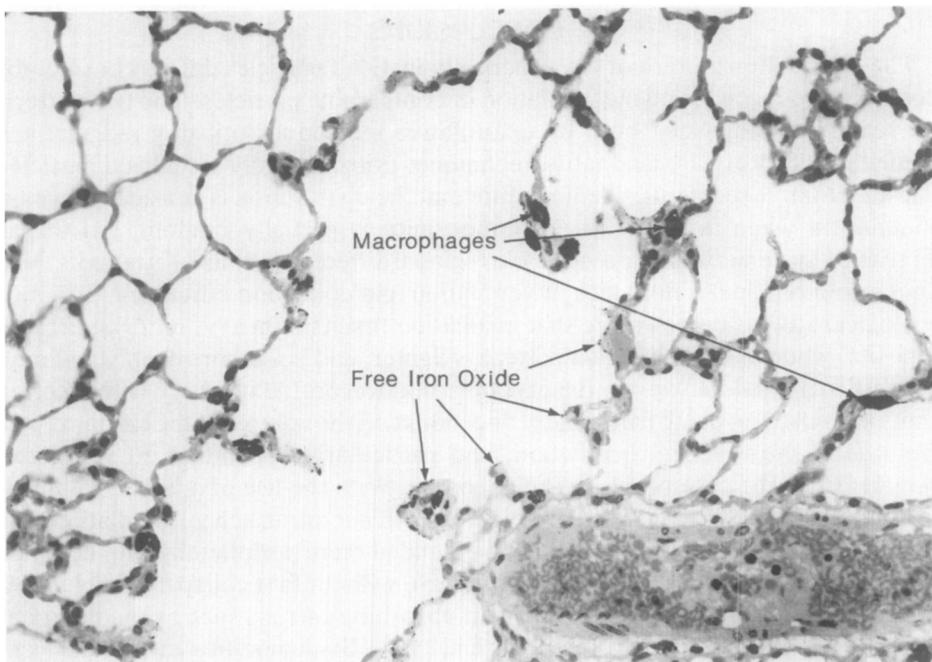


FIG. 9. Inhalation exposure of hamster lung, 1 hour after exposure, 4 hours after onset. Particle deposition delicate and widespread, not completely uniform. Many macrophages engaged in phagocytosis. Prussian blue and basic fuchsin. $\times 250$.

Lobes from four of these cat lungs were subsequently lavaged and their alveolar macrophages recovered. Although much of the carbon had been ingested by alveolar macrophages, the carbon was distributed very unevenly among the cells. In most cases, 80 to 90% of the cells were completely free of carbon. The remaining cells were engorged with carbon particles. In addition, large quantities of extracellular carbon were present. The observations contrast with those obtained from cats exposed to carbon aerosols by inhalation. Although there was still considerable variability in the amount of material ingested per macrophage, more than 80% of the cells contained at least some particles. We infer that the nonuniform distribution of particles within alveolar macrophages stems from the uneven distribution of material instilled through the trachea. It is also possible that instilled material may plug up whole alveoli or small airways, and thus some of it may be rendered temporarily inaccessible to the macrophages.

Other potentially important morphological concerns have not been examined here, but should be considered by the reader. For example, it is possible that the intratracheal instillation procedure (especially in unskilled hands) injures the tracheal mucosa, alters the quantity and quality of the secretions, and thereby alters the mucociliary transport system. It is also possible that the instillation medium may alter the alveolar environment or the integrity of the air-blood barrier. Surfactants, such as Tween 20 or 80, are often present in commercially available suspensions of small particles. In adequate concentrations, such surfactants may provoke pulmonary edema. Depending on the purposes of the experiment, these possible artifacts should also be considered.

CONCLUSIONS

The distribution pattern of intratracheally instilled particles differs considerably from that produced following inhalation of comparable particles. The two patterns are readily distinguished both by quantitative techniques utilizing radioactively labeled particles and by qualitative techniques utilizing easily visualized particles. The quantitative techniques demonstrate that the distribution is considerably more nonuniform when instilled. The nonuniformity is partially random, but in part represents systematic and reproducible regional differences caused by gravitational forces, and regional differences in ventilation and collection efficiency. The morphological studies demonstrate that instillation results in heavy, more centralized deposits, whereas the inhalation pattern is lighter, and both more evenly and more widely distributed. However, the exact distribution profile will vary within certain limits in each case, being particularly dependent on the volume of the carrier fluid in the case of intratracheal instillation, and particularly dependent on ventilatory parameters in the case of inhalation exposure. With the use of a larger volume of carrier than the 0.15 ml/100 g body weight used in our intratracheal instillations, the centralized region of exposure could be extended more peripherally, but complete filling of the lungs would be difficult to achieve without first collapsing the lungs or substituting for saline a more effective air absorbing carrier, such as the fluorocarbon perfluorobutyl tetrahydrofuran (Boren, 1968). Such modifications of the instillation technique might well lead to improvements in the uniformity of particle deposition throughout the lungs, but they would be at the cost of complicating a desirably simple and relatively harmless procedure.

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