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Clearance from the Respiratory Tract[1]

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Clearance from the Respiratory Tract. SCHLESINGER, R. B. (1985). *Fundam. Appl. Toxicol.* **5**, 435–450. Clearance is an essential component of the defense system of the respiratory tract. Alterations in the clearance of insoluble particles have been shown to provide a sensitive indicator of response due to exposure to various inhaled materials. This paper reviews clearance mechanisms, the significance of clearance changes, and the methodology used to assess them.

Clearance is the physical removal out of the respiratory tract of material which deposits on airway surfaces. It is a major component of a defense arsenal which in total serves to prevent or reduce both local damage by and systemic absorption of inhaled toxicants.

Examination of the effects of inhalants upon lung defense systems should be a part of overall toxicological assessments, since regardless of the specific protocols of any inhalation study, determination of the fate of these materials is essential for interpretation of results obtained from the exposures. The rates and routes of clearance relate to the residence time of deposited agents within the respiratory tract and it is ultimately this time which, when coupled with aspects of metabolism and physicochemical properties of the inhalant, relates to the degree of local and/or systemic toxic response.

This paper describes the use of techniques designed to measure changes in the clearance of insoluble, nonviable particles from the respiratory tract of experimental animals and humans as an endpoint in inhalation toxicological studies, i.e., as an indicator of toxic response and/or as a predictor of respiratory tract disease.

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OVERVIEW OF CLEARANCE MECHANISMS AND PATHWAYS

Clearance comprises various physiological functions, and is not a uniform process throughout the entire respiratory tract. There are regional differences, both mechanistically and temporally.

Clearance of insoluble material from the conducting airways occurs via the mucociliary system. Except for the vestibular region of the nasal cavity and the posterior nasopharynx, the nasal passages and the tracheobronchial tree through the terminal bronchioles are lined with a ciliated epithelium overlaid by a fluid, commonly called mucus.

In the upper respiratory tract and the bronchi, the fluid blanket has two distinct layers (Lucas and Douglas, 1934), a low-viscosity hypophase, which surrounds the cilia and within which they move, and a high-viscosity epiphase lying on top of the cilia. In bronchioles, the fluid layer lacks the epiphase and is thinner (Luchtel, 1976); its composition and structure resemble that of surfactant (Macklem *et al.*, 1970; Gil and Weibel, 1971). Deposited material is cleared from most conducting airways by movement of the epiphase due to the coordinated beating of the cilia; movement of mucus from the nasal passages occurs distally toward the nasopharynx, and from the bronchial tree proximally toward the oropharynx. In the bron-

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chioles, fluid movement is likely due both to ciliary beating as well as to surface tension differences between bronchioles and alveoli. The nasal vestibular region is cleared via mechanical means, i.e., blowing, wiping, or sneezing.

Clearance from the nonciliated respiratory region of the lung occurs via a number of mechanisms and pathways, but the relative importance of each is not always certain and may depend to some extent upon the physicochemical properties and amount of material deposited. The first line of defense is the alveolar macrophage. These large cells move on the alveolar epithelium via amoeboid motion, are phagocytes, and contain an array of proteolytic enzymes that allow them to digest a wide variety of materials. Contact with deposited particles may occur by chance, or be due to directed motion resulting from the release of chemotactic factors. Although most deposited particles are ingested, which helps prevent their penetration through alveolar epithelium and translocation to other sites, the efficiency of phagocytosis may depend on specific properties of the particles, e.g., size, shape, and composition (Camner, 1980).

Particle-laden macrophages, as well as free particles, may be cleared from the respiratory region via a number of pathways, one being the mucociliary system. However, the route(s) by which macrophages reach the distal end of the mucous blanket is uncertain. Suggested pathways are along the alveolar surface, due to surface fluid flux or directed locomotion (Kilburn, 1968; Scarpelli and Condorelli, 1975; Sorokin and Brain, 1975; Ferin, 1976), or via the pulmonary interstitium (Brundelet, 1965; Green, 1973; Kilburn, 1974; Tucker *et al.*, 1973), as cells sieve through the alveolar epithelium into interalveolar areas, from which they then penetrate into the bronchiolar lumen.

Although it is not certain whether macrophages can actually reenter the interstitium from alveolar spaces, free particles may be directly translocated through the alveolar epithelium (Lauweryns and Baert, 1974; Robertson, 1980; Brody *et al.*, 1981). Once there, these particles may be engulfed by resident macrophages which, in turn, may migrate to a nearby lymphatic channel or be carried in the flow of interstitial fluid toward the lymphatic system, bronchial tree, or to perivenous or subpleural sites.

Uningested particles in the interstitium may traverse the capillary endothelium (Robertson, 1980), while extremely small particles, i.e., those with diameters < 10 nm, may enter the blood directly from alveoli via diffusion through the air–blood barrier (Gross and Westrick, 1954; Raabe, 1982); however, entry into the lymphatic system is more likely. Clearance via the lymphatic system is relatively slow (Ferin, 1976; Sorokin and Brain, 1975), and particles may be trapped in bronchial lymph nodes, which often become major reservoirs of retained materials.

Soluble particles can dissolve in the alveolar fluid, then diffuse through the epithelium and interstitium into the lymph or blood (Chinard, 1966; Morrow, 1973). Although dissolution competes with other clearance mechanisms for temporal advantage in all respiratory tract regions, because the mechanical clearance processes in the alveolar region are slow, materials considered to be relatively "insoluble" may have high rates of dissolution *in vivo*, especially if the particle size is very small (Raabe, 1982). Unfortunately, the factors affecting solubility are poorly understood, although it is known to be influenced by surface–volume ratio and other surface properties of deposited particles (Morrow, 1973).

In terms of kinetics, clearance from the respiratory tract occurs at different temporal scales in different regions and, to some extent, even within the same region. For example, the velocity of the mucus blanket increases from distal to proximal airways in the bronchial tree. Insoluble material that deposits and remains on ciliated airways will normally be cleared from the respiratory tract within 24 hr; the exact time depends upon the depth of penetration. However, the completeness of bronchial clearance within this time has

occasionally been challenged, and it has been suggested that slower clearance from peripheral bronchi may extend beyond this 24-hr period (Clarke and Pavia, 1980) and that a small fraction of deposited material may actually be retained within the bronchial tree for days (Patrick and Stirling, 1977a).

Particles depositing in respiratory airways generally remain longer than those deposited in conducting airways. Casarett (1972) has related a series of three temporal phases for normal alveolar clearance to specific physiological processes: an initial fast phase, having a half-time of ~2–6 weeks, representing relatively rapid clearance by macrophages via the bronchial tree; an intermediate phase of slower macrophage-mediated clearance, involving translocation via the lymphatic channels, and having a half-time on the order of months; and a phase of still slower clearance, with a half-time of months to years, representing removal by dissolution.

SIGNIFICANCE OF CLEARANCE AS A TOXICOLOGICAL ENDPOINT

A valid toxicological endpoint should meet certain basic criteria. It should be reproducible (in controls), it should be fairly sensitive to change following exposure to appropriate agents, and it should have some relationship to respiratory tract disease.

Both alveolar and mucociliary clearance rates are well-defined functional characteristics of an individual when tests are performed under the same conditions. There is a close correspondence in repeated analyses in the same individual for alveolar clearance rates in, for example, humans (Bohning *et al.*, 1982), dogs (Gibb and Morrow, 1962), and donkeys (Halpern *et al.*, 1981), and for mucociliary clearance rates in humans (Lippmann *et al.*, 1980), donkeys (Schlesinger *et al.*, 1978, 1979), and rabbits (Chen, 1983).

Work performed in this laboratory has shown bronchial mucociliary clearance alteration to be a sensitive physiologic indicator of irritant response, based upon acute exposures to one particular material, sulfuric acid (H_2SO_4) mist, a common ambient air pollutant. Transient alterations in bronchial mucociliary rate have been produced at low exposure levels in humans (0.1 mg/m^3) (Leikauf *et al.*, 1981), donkeys (0.2 mg/m^3) (Schlesinger *et al.*, 1978), and rabbits (0.1 mg/m^3) (Schlesinger *et al.*, 1984). Although such a response to inhaled irritants may be adaptive, helping to maintain organ homeostasis, these changes are more likely a pathophysiological response of the airways and, although temporary, they may foreshadow more permanent alterations or progressive changes which would follow continued exposures (Schlesinger *et al.*, 1978, 1979).

Interpretation of mucociliary clearance alterations in terms of potential health problems is somewhat speculative. Dysfunction of mucous transport may be involved in the pathogenesis of both acute and chronic respiratory disease, but the clinical relevance of mucociliary change is only recently being elucidated and its importance to pulmonary disease development is only beginning to be experimentally established (e.g., Schlesinger *et al.*, 1983). Clinical studies of individuals with a disease syndrome characterized by impaired clearance, i.e., primary ciliary dyskinesia (PCD), may be used to assess the importance of mucociliary transport and the effect of its dysfunction upon respiratory disease, and to provide information on the role of mucociliary clearance in maintaining the integrity of the lung. The lack of mucociliary function in PCD is directly responsible for the early development of recurrent respiratory tract infections and, eventually, chronic bronchitis and bronchiectasis (Wanner, 1980; Rossman *et al.*, 1984). It is, however, not certain whether partial impairment of the mucociliary system will increase the risk of lung disease. In this regard, the rate of mucociliary clearance may be a factor affecting the development of infectious disease. Pathogenic organisms depositing in the conducting airways are confronted with the mucociliary barrier, and the extent of penetration of vectors through the mucus to the underlying

cells may be important in disease development. This rate, relative to the rate of mucociliary transport out of the respiratory tract, could determine the effectiveness of inhaled pathogens in initiating disease (Proctor, 1979; Niederman *et al.*, 1983). It is known that in conditions characterized by retarded clearance from conducting airways, e.g., chronic bronchitis, there is a predisposition to respiratory infection (Cumming and Semple, 1980). In addition, destruction of the functional integrity of the ciliated epithelium can result in impaired defense against bacteria (Laurenzi and Guarneri, 1966), and impaired transport has been observed in viral respiratory infections (Bang and Foard, 1964; Lourenco *et al.*, 1971b).

Retardation of mucociliary clearance may also be a factor in the pathogenesis of bronchial cancer. Inhaled aerosols tend to selectively deposit at airway branching sites in the bronchial tree (Schlesinger and Lippmann, 1978). Histological studies (Kotin and Falk, 1959; Auerbach *et al.*, 1961) have indicated that neoplastic and preneoplastic lesions predominate at these bifurcation regions. Macklin (1956) and Hilding (1957) have suggested that bifurcations are areas of normally slower mucus movement; this would add to the residence time of particulates which deposit within these areas, or are being carried through them on the mucociliary escalator. Thus, the selective distribution of lesions at bifurcations within the upper bronchial tree may be the result of both selective deposition and slowed clearance, resulting in prolonged retention of high local concentrations of deposited carcinogens. Although there is no direct evidence that ineffectual clearance is a contributory factor to the development of bronchogenic carcinoma, a causal relation has been suggested between adenocarcinoma and sites of local particle retention and inadequate clearance in the nasal passages of furniture workers (Morgan *et al.*, 1974; Hadfield and Macbeth, 1971).

There is accumulating evidence that dysfunction of bronchial clearance plays a role in the pathogenesis of chronic bronchitis; it is known that mucus transport is impaired in individuals who have this disease (Wanner, 1977). The rate at which individual healthy humans clear deposited particles from the lungs varies widely. In cigarette smokers and persons with chronic obstructive pulmonary disease, there is a somewhat wider variation (Albert *et al.*, 1973; Gongora *et al.*, 1981). In such groups, the within-subject variation is also greatly increased, suggesting that loss of control of mucociliary transport could cause and/or result from such disease. Schlesinger *et al.* (1983) showed, in an experimental animal, that modest changes in mucociliary clearance rates may be associated with secretory epithelial changes in small bronchi and bronchioles, changes which, if continued, could lead to clinical manifestations of bronchitis. Other studies suggest that mucociliary dysfunction is an early indication of disease changes in the lungs. For example, retarded clearance has been demonstrated in bronchitics who showed no sign of airway obstruction (Mossberg and Camner, 1980), while young smokers having various degrees of impairment of tracheal mucus transport rates had no overt bronchitic symptoms and had normal pulmonary function (Goodman *et al.*, 1978).

The pathogenic implications of alterations in clearance from the alveolar region have not been examined to the extent that changes in mucociliary clearance have. Alveolar clearance rates appear to be reduced in people with chronic obstructive lung disease (Bohning *et al.*, 1982) and in cigarette smokers (Cohen *et al.*, 1979; Bohning *et al.*, 1982), suggesting some relation between altered defense and disease development. Clearance dysfunction has also been shown in experimental animals having viral infections (Cresia *et al.*, 1973).

The adequate performance of alveolar macrophages is critical in determining the effectiveness of pulmonary region defense in minimizing the residence time of deposited toxicants. For example, phagocytosis plays an important role in the prevention of particle entry into fixed tissues of the lung, a region

from which clearance is very slow; accumulations of several types of dust have been directly linked to development of lung disease. Damage to macrophages has also been implicated in the pathogenesis of chronic lung diseases involving proteolysis (e.g., emphysema) and fibrogenesis (e.g., silicosis, asbestosis) (Brain, 1980; Warheit *et al.*, 1984), as well as in an increased risk of viral and bacterial infections (Hocking and Golde, 1979). Furthermore, the viability and functional activity of macrophages is impaired in people with asthma (Godard *et al.*, 1982).

TECHNIQUES OF CLEARANCE MEASUREMENT

Choice of Animal Model

The species of choice in clearance studies is humans, but certain experimental protocols preclude their use. Fortunately, the broad mechanisms of clearance, i.e., mucociliary in conducting airways and cellular and dissolution in respiratory airways, are similar in humans and other mammals commonly used in inhalation toxicology studies. Interspecies differences do occur, for example, in secretory cell morphology and distribution, macrophage function, the relative role of each clearance mechanism, or rates and efficiency of clearance from various regions, differences which could affect retention and dose (Brain and Mensah, 1983). Short-term, i.e., mucociliary, times especially may show a substantial difference between species, even for similar particles deposited under comparable exposure conditions; much greater interspecies similarity occurs in patterns of clearance from aerosols deposited distally to ciliated airways (Thomas, 1972; Phalen, 1984).

Although rates of mucociliary clearance may be species specific, the response to numerous toxicants, e.g., H_2SO_4, SO_2, and cigarette smoke, is qualitatively similar between humans and many experimental animals (Lippmann *et al.*, 1982; Schlesinger *et al.*, 1984; Phalen, 1984). Thus, one particular species may not necessarily be more suitable than another as a model for clearance studies. Although there are anatomical and physiological differences, these may be secondary to a project whose endpoint is alteration of clearance due to an inhaled toxicant. Specific animals, thus, may be chosen for use based upon other criteria. For example, accurate measurements over extended periods of time favor the use of unanesthetized animals, and are facilitated by the use of species which are relatively docile.

Specific Techniques

Clearance studies with humans or experimental animals are generally performed by examining the rate of removal or transport of markers from the lungs as a whole, or from specific individual airways. When using experimental animals, serial sacrifice or fecal analysis techniques may be employed. The actual measurements of rates or times are strongly influenced by the specific methodology.

Measurements of Local Mucus Velocity

Mucus transport velocities in the nasal passages, trachea, and main bronchi may be directly measured by monitoring markers on the epithelium, or by measurement of the movement of boli of radioactive particles selectively deposited within these airways or moving through them from more distal areas. Actual rates of movement are obtained by determining the time needed for the bolus or marker to traverse a calibrated distance or to move between two anatomically defined areas. The markers used have included cellular debris, or material specifically introduced into the airways, e.g., india ink droplets, Teflon disks, radiolabeled resin beads, powders, colored solutions and dyes, or pollen grains.

Various measurement techniques have been used. Proctor *et al.* (1977) placed sac-

charin particles in the nose of humans and measured the time until the subject reported the first taste of sweetness. Some nasal markers have been viewed directly, or indirectly by looking, with mirrors, for their appearance in the nasopharynx after placing them on the anterior nasal mucosa (van Ree and van Dishoeck, 1962; Bang *et al.*, 1967). Radioactive or radioopaque markers in the upper respiratory tract or central airways may be viewed using external monitoring techniques, including fluoroscopy (Friedman *et al.*, 1977; Goodman *et al.*, 1978; Mezey *et al.*, 1978), cinebronchofiberoscopy (Sackner *et al.*, 1973; Santa Cruz *et al.*, 1974; Landa *et al.*, 1975; Toomes *et al.*, 1981), scintillation detection (Proctor *et al.*, 1977; Patrick and Stirling, 1977b; Man *et al.*, 1980; Wolff *et al.*, 1982b), or gamma-camera imaging (Quinlan *et al.*, 1969; Chopra *et al.*, 1977; Puchelle *et al.*, 1982; Wolff *et al.*, 1982b).

Transport velocities in the trachea and main bronchi have also been assessed by monitoring the movement of inhaled boli of radiolabeled particles. Yeates *et al.* (1975) selectively deposited a bolus of large, tagged albumin microspheres in human central airways. Movement along the trachea was monitored by a gamma camera. Later techniques employed a multidetector probe consisting of a series of three or six small collimated scintillation detectors aligned over the trachea, and boli of tagged ferric oxide microspheres (Yeates *et al.*, 1981a,b). Foster *et al.* (1978; 1982), using a gamma camera, assessed the transport velocity in the major bronchi of labeled ferric oxide. Wolff *et al.* (1982b) measured the leading edge of instilled, tagged albumin in the trachea of rats using scintillation detectors, and in dogs with a gamma camera. Tracheal mucus velocities have also been measured in donkeys by assessing the time required for a bolus of tagged particles to traverse a fixed distance between two tracheal fields as these particles moved up from distal airways (Schlesinger *et al.*, 1978).

The advantage of local velocity techniques is that they allow measurement in anatomically defined, albeit limited, airways. In addition, because of the use of a specific site, there is no question as to whether altered clearance rates due to toxicant exposure resulted from actual alterations in the mucous system, or to a change in deposition pattern; the latter is a possibility when using whole-lung clearance assays, as discussed below. However, there are a number of disadvantages. The marker techniques are invasive, in that particles are usually selectively introduced into the airway of interest. The necessary use of anesthetics may affect the observed transport rates (Landa *et al.*, 1975; Patrick and Stirling, 1977b), and the actual method of introduction may result in some trauma to the airway. In addition, if only one or two marker particles are used, placement is critical, since zones of slower and faster transport may occur in the airways (Phipps, 1981). It is also possible that the physicochemical properties and, perhaps, size of the marker may determine the extent of penetration into the mucus and, therefore, the manner in which it is transported (Lee *et al.*, 1979; Wolff and Muggenburg, 1979). However, a few studies have shown that the rate of tracheal transport of one material in various animals or of various materials in one animal was not influenced by the character of the marker, i.e., its size, mass, or composition (Antweiler, 1958; Man *et al.*, 1980). Ahmed *et al.* (1979) suggested that radiation from the tracer particles may affect transport rate, but a later study by Wolff *et al.* (1982a) did not support this.

Because it is easier to measure than is whole-lung clearance, alteration in tracheal transport rate has been used as the sole endpoint in a number of studies aimed at assessing the effects of inhaled toxicants upon the mucociliary clearance system. However, such transport velocities may not be adequate indices to imply an overall effect. For example, studies performed in this laboratory in both humans (Leikauf *et al.*, 1981) and donkeys (Schlesinger *et al.*, 1978, 1979; Albert *et al.*, 1974) demonstrated irritant aerosol-

induced changes in bronchial clearance that were not associated with any alteration in tracheal transport rate.

The reason for the lack of effect upon tracheal transport in these studies may be that the trachea is more resistant to irritant effects or, more likely, the small size of the irritant aerosols used precluded significant tracheal deposition. This latter is supported by Wolff *et al.* (1981), who reported a depression in tracheal transport rate in anesthetized dogs exposed to a 0.9-μm H_2SO_4 mist while no effect was observed at identical exposures to a 0.3-μm aerosol. With exposures of sheep to even higher mass concentrations, Sackner *et al.* (1978) found no change in tracheal mucus velocity when using a 0.1-μm H_2SO_4 aerosol. Thus, the use of alterations in clearance in specific airways as a monitor of mucociliary function is valid only if enough of the agent of interest deposits within the region measured. Therefore, care must be exerted in selecting regions of interest based upon the properties of the toxicant employed in the study.

Whole-Lung Clearance

Radioaerosol technique. The most commonly used technique to measure whole-lung clearance involves inhalation of a radiolabeled tracer aerosol. The total amount of radioactivity remaining in the lung at selected intervals is then measured by external detector systems. The decline in emission rate, corrected for radioactive decay, represents clearance. Tracer materials used have included Teflon, polystyrene latex, hematite, magnetite, clay, and albumin.

Various types and configurations of scintillation detectors have been used to monitor clearance; these may be divided into profile scanning (mobile) or stationary (fixed) systems. In the former arrangement, either (1) the detectors move relative to the thorax of the subject who has inhaled the tracer aerosol (LaBelle *et al.*, 1964; Holma, 1967a,b; Camner *et al.*, 1971; Camner and Philipson, 1971, 1978), or (2) the subject is moved relative to stationary detector(s) (Albert *et al.*, 1968). In either case, measurements are obtained in discrete preselected positions, or during continuous scanning, i.e., movement at a constant rate.

Scanning systems are relatively independent of the apex to base distribution of deposited tracer particles in the lung, since they provide a longitudinal activity distribution map. They also reduce counting variations due to slight differences in positioning the subject or due to subject movements, since they are not as dependent upon the exact equivalent geometry of the subject's location in relation to the detectors as are fixed systems.

In fixed systems, collimated detectors are placed in one or more positions relative to the subject's thorax. Configurations used include single, central anteriorly placed detectors (Albert and Arnett, 1955; Toigo *et al.*, 1963; Thomson and Short, 1969); two detectors, one placed centrally and one laterally (Luchsinger *et al.*, 1968); twin axially opposed detectors placed anterioposteriorly (Booker *et al.*, 1967; Thomas *et al.*, 1974; Leikauf *et al.*, 1981) or laterally (Schlesinger *et al.*, 1982b); and multiple detector arrays (Albert *et al.*, 1969; Stahlhofen *et al.*, 1981; Bailey *et al.*, 1982). Additionally, two other systems have been used. These are the gamma camera (Klimek *et al.*, 1969; Lourenco *et al.*, 1971a; Sanchis *et al.*, 1972; Wilkey *et al.*, 1980; Puchelle *et al.*, 1982) and the whole-body counter (Cresia *et al.*, 1973; Bohning *et al.*, 1982; Snipes *et al.*, 1983). Most fixed systems provide for measurements in only one subject at a time. A system developed by Schlesinger *et al.* (1982b) for rabbits allows simultaneous measurement in up to 15 animals.

Unilateral detector systems require exact positioning for reproducible results, while dual or multidetector systems (where the signal output is combined) are less sensitive to changes in position of the subject in the measurement plane or to effects of redistribution of retained tracer particles in the

lungs. The gamma camera provides an assessment of total clearance and also allows actual visualization of the distribution of retained particles at various times postexposure. The imaging field is usually divided into central and peripheral areas, although more recent techniques subdivide the lung further.

The airways within which the test aerosol deposits exist in a three-dimensional array in the lungs and are, therefore, at various depths relative to the detectors. Thus, the efficiency at which retained activity within each airway is measured will vary. It therefore follows that the detector configuration will affect the ultimate shape of the clearance curve. In addition, because of sensitivity differences, the amount of activity needed for a successful study varies, depending upon the type of detectors used. The gamma camera offers the greatest advantage in terms of spatial resolution but it has low sensitivity and requires large amounts of activity. Scanning systems have poorer spatial resolution but better sensitivity, while multiple stationary detectors, which offer little information on intrathoracic particle distribution, are the most sensitive. The most responsive fixed system is the whole-body counter. However, this technique is not suitable for use during the first few days after tracer exposure since, because it is not collimated, it cannot effectively distinguish between activity in the lungs from that cleared into the stomach during the initial, rapid tracheobronchial clearance phase. It can, however, be used to monitor long-term clearance once the activity in the rest of the body is low compared to that in the lungs.

Normal clearance patterns which are carried out beyond 1 day have two distinct broad temporal phases: an initial rapid phase, and a subsequent slower phase. The former is presumed to represent mucociliary clearance of aerosol initially deposited on the bronchial tree, while the latter is controlled largely by nonmucociliary mechanisms, and presumably represents material deposited below the level of the mucous escalator. Since regional deposition is not directly measured, these apportionments are based on the assumption that material cleared within 24 hr after inhalation resided initially on the bronchial tree. Therefore, radioactivity remaining after this time represents material in the alveolar region. Although the assumption is a useful one, it has occasionally been challenged, as discussed previously.

Quantitation is necessary to describe the effects of toxicants, and clearance curves have been analyzed in a number of ways. They have been fitted with single exponentials, or a series of such functions. However, because all clearance mechanisms occur simultaneously, the terms in such functions may not necessarily be related to specific physiologic mechanisms. Curves have also been described in terms of the fraction or percentage of initial lung burden which remains at various times postexposure or by the time required for a certain percentage of this initial activity to be cleared. Total clearance may also be assessed by measuring the area under the clearance curve. Gamma-camera images are analyzed in terms of changes in the distribution of particles in the various lung zones.

One of the major problems associated with external monitoring techniques is the dependence of the observed mucociliary clearance pattern upon the pattern of initial deposition of the tracer aerosol. This is because the techniques are indirect, and clearance parameters are proportional to transit pathways. Thus, for example, an apparent increase in clearance rate after toxicant exposure could be due to a proximal shift in deposition of the tracer aerosol rather than to an effect on the clearance system itself. This may be a special problem when comparing different groups, e.g., healthy subjects and those with chronic obstructive lung disease, since the latter may tend to have greater central deposition than the former for the same size tracer aerosol (Lippmann *et al.*, 1980).

The influence of aerosol size upon the pattern of bronchial clearance of a tracer

aerosol after irritant exposures was shown by Lippmann *et al.* (1981). This study indicated that measurement of the clearance rate of a single monodispersed tracer aerosol may not be adequate for simultaneously assessing effects of a toxicant on all conducting airways, and that the deposition pattern of the tracer aerosol can make an important difference in the apparent effect of the toxicant on mucus transport. This type of problem may be avoided by exposure to two different sized tracer aerosols incorporating different tags to allow monitoring of clearance from both the upper and lower bronchial tree, simultaneously. This technique would also provide a more precise localization of the site of action of a toxicant on the lungs.

Another potential problem in analyzing bronchial clearance is the effect of coughing. Coughs may produce a stepwise decrease in the amount of tracer particles left in the lung, and must be taken into account when assessing clearance times due solely to mucociliary transport (Camner *et al.*, 1973; Sanchis *et al.*, 1973).

The shapes of mucociliary clearance curves are highly dependent upon tracer particle deposition. In studies of alveolar clearance, however, different clearance rates may also occur when using different sized particles (Bailey *et al.*, 1982), since there may be size-dependent differences in macrophage phagocytosis, *in vivo* solubility, etc. Differences in the long-term clearance of equivalent-sized particles but consisting of different materials have been noted by Stahlhofen *et al.* (1981) in humans, and by Schlesinger *et al.* (1982a) in donkeys.

Because of wide differences in techniques, comparisons between studies of different investigators is difficult, and lung-clearance percentages and times may not be comparable. Nevertheless, clearance studies with radiolabeled tracers have been successfully used to demonstrate dysfunction following exposure to numerous inhaled agents or due to disease (Schlesinger *et al.*, 1984; Last, 1982; Wanner, 1977). These techniques allow clearance to be measured repeatedly in the same animals so fewer numbers are needed, and the use of each animal as its own control in many studies markedly reduces the variability inherent in comparisons between individuals.

Magnetopneumography. The assessment of alveolar clearance requires measurements to be performed over perhaps several months. When using radioactively tagged tracer aerosols a nuclide having a relatively long half-life is required. In addition, since the total dose to the subject should be minimized, especially if humans are used, long counting times may be required to obtain statistically reliable data. Thus, very-long-term clearance studies using humans may preclude use of radioisotopic tracers due to potential health risks.

A relatively new technique which avoids these problems is magnetopneumography (MPG). The measurement of magnetic fields due to contaminants in the lung was first reported by Cohen (1973). Based upon this work, controlled exposures to an inert, magnetic dust (magnetite, Fe_3O_4) have been performed in humans and experimental animals to assess clearance (Cohen *et al.*, 1979; Valberg and Brain, 1979; Halpern *et al.*, 1981; Freedman and Robinson, 1981). The subject is exposed to the aerosol and, at various times, a magnetic field is applied externally to the thorax; the field must be of sufficient strength and be applied for an appropriate length of time to magnetize the bulk of the deposited particles. Two magnetization techniques have been employed. In uniform-field MPG, the whole lung is magnetized. In localized-field MPG, one region is magnetized at a time and the resultant field is measured and then erased prior to application of the magnetic field to the next region. The latter technique helps avoid internal cancellation of fields which may occur when the entire thorax is magnetized at once (Robinson and Freedman, 1979).

After the magnetizing field is removed, a remanent field remains. The subject is moved

toward and then away from a magnetic sensor, which is positioned at a specific location relative to the chest; the change in field strength as measured by the sensor is a reflection of retained particles in the measured region of interest. This method of differential measurement is necessary because strong, low-frequency variations of the local field often make it impossible to perform continuous measurements of the remanent chest field. Because the lung remanent field is much weaker than the earth's magnetic field or field variations due to environmental sources, measurements must be performed in a magnetically shielded chamber or with a gradiometer-type field sensor, which reduces sensitivity to both uniform background fields and local fluctuating fields. Specific sensors range from large, superconducting devices (SQUID) to table-top fluxgate magnetometers.

Magnetopneumographic techniques have some advantages over radioaerosol techniques in terms of both temporal resolution and spatial resolution in the measurement plane. However, there are a number of significant problems. All sources of external magnetic contamination on the subject must be removed. The mobile units used to move the subject toward the sensor must be constructed of nonmagnetic material. In addition, there are difficulties in deducing actual particle distribution in the lungs from the data, since critical positioning is required; depending upon field size, a slight misalignment can result in a dramatic change in the results. This is because the magnetic-field measurements are much more sensitive to tracer particles in the lung periphery, which is closer to the probe, than to dust in other areas. Thus, material translocated toward the pleural surface of the lung as part of the clearance process would result in an increase in the observed magnetic field, while translocation from this area would result in a field decrease. Because of the sensitivity of the remanent magnetic-field measurements to the actual distribution of retained particles within the effective viewing window, considerable caution must be exercised in directly interpreting field data in terms of lung clearance. Use of localized field MPG with partial erasure may provide information on particle depth, and permit a distinction between internal translocation and clearance (Freedman *et al.*, 1982).

Magnetic techniques do have potential advantages in that certain information may be obtained using them which is not obtainable by other whole-animal *in vivo* techniques. When the external field is applied, the resulting magnetic moment increases rapidly at first, then more slowly (the moment can be deduced from the initial remanent field measurement). This time dependence is affected by the viscosity of the medium in which the deposited particles reside (Williamson and Kaufman, 1981). Therefore, as free particles are translocated from alveolar surfaces or engulfed by macrophages, the response to an externally applied field may change. Thus, when magnetic measurements are used to assess clearance over a long time period, it is possible that some or all of any observed decline in remanent moment is due to immobilization of particles. In addition, the hysteresis curves of magnetization as well as relaxation (loss of magnetic alignment) may provide information on the amount of fibrosis in lung tissue (Cohen, 1975).

The validity of magnetopneumographic techniques as a monitor of clearance was shown in this laboratory by Halpern *et al.* (1981). In this study, donkeys inhaled magnetite which had been neutron activated to $^{59}Fe_3O_4$, so that concurrent measurements using a proven, radiologic technique could be performed. The paired radiologic and magnetic curves displayed definite changes in slope at approximately the same times postexposure, as well as similar rates of decrease with time. This confirmed that the reduction of the initial remanent field over several months represented particle clearance and was not due simply to a chemical change of the iron compound to a nonmagnetic form or to dissolution, with loss of magnetic properties.

Bronchographic technique. One technique used for whole-lung clearance in both humans and experimental animals allows visualization of tracer particle distribution without the need for radiolabeled aerosols. This involves insufflation of radioopaque tantalum powder through a tracheal catheter. Serial chest X rays are then performed over a period of months to provide a visualization of clearance, which is quantitated based upon visual scoring of film intensity (Gamsu *et al.*, 1973; Wood *et al.*, 1973). Although the technique provides some measure of whole lung and regional clearance patterns, and allows measurement in anatomically defined airways, it is invasive, only semiquantitative, and requires high radiation doses, making it unsuitable for routine use in humans. In addition, it requires several grams of tantalum, which may overload clearance systems. This technique, therefore, has not found widespread use.

Fecal analysis technique. A technique for indirect monitoring of clearance which involves radioaerosols but not external monitoring is fecal analysis (LaBelle and Brieger, 1959; Kenoyer *et al.*, 1981; Mannix *et al.*, 1983). This procedure involves collection of feces at fixed intervals after exposure to a radiolabeled aerosol. The fecal excretion activity curve presumably represents material cleared via the mucociliary system into the gastrointestinal tract and can thus be used to provide an index of tracheobronchial clearance. It makes the assumption that all material cleared from the lung is transported to the gastrointestinal tract and excreted in the feces. This technique is very sensitive to feeding behavior of the animals; those that do not eat or do not excrete for a particular fraction of the sampling intervals cannot be included in the analysis.

Spritzer *et al.* (1971), and Spritzer and Watson, (1964) employed an esophageal collection method in rats to monitor bronchial clearance. At various times postexposure, a collection tube placed in the esophagus and which exited through the abdominal wall was flushed, and the activity counted in the washes to provide clearance rates, i.e., particles cleared per collection interval. The relation between clearance rates as measured in such surgically altered animals and normal clearance rates is not known.

Serial sacrifice technique. Clearance of deposited particles may be monitored in experimental animals by serial sacrifice at various intervals after exposure, followed by analysis of material in various parts of the respiratory tract or in the lungs as a whole. The lung burden plotted as a function of time provides an assessment of clearance.

The method of analysis is dependent upon the nature of the material used. Techniques employed have included gamma-ray spectrometry for neutron-activated or radiolabeled particles (LaBelle and Brieger, 1959; Reznick and Borgmeyer, 1980; Wehner and Wilkerson, 1981; Sweeney *et al.*, 1983); autoradiography (Sweeney *et al.*, 1983); light and/or electron microscopy (Sorokin and Brain, 1975); and chemical analyses (Ferin *et al.*, 1983).

Sacrifice techniques have the advantage of being very sensitive, i.e., they have the potential to detect very small amounts of material retained in the lungs, using appropriate analytical techniques. Although microscopic techniques generally provide only qualitative assessments of particle distribution and clearance from various regions, other techniques may allow quantitative determination of the amounts of material retained in different regions of the respiratory tract, and without interference from material in adjacent areas. The major disadvantages are that a large number of animals are needed for statistical reliability, intraindividual variability in clearance cannot be examined, and it is not possible to examine the effects of toxicants upon the course of clearance in the same individual on different occasions.

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