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Dust Overloading of the Lungs: Update and Appraisal

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This article reviews recent studies which involve, or impact on, the condition of dust overloading in the lungs of several species, especially the Fischer 344 rat. Its main purpose is to provide an update of the overload concept and new information of possible mechanistic relevance. At present, the most likely general explanation for the suppression of particle transport by the alveolar macrophage (AM) and the development of concurrent events, e.g., increased interstitial dust uptake and prolonged inflammatory response, is the persistent, possibly excessive, elaboration of chemotactic and chemokinetic factors by the AM. The induction of these interrelated events is hypothesized as related to the volume of dust phagocytized by the AM pool. The review concludes, *inter alia*, that information is badly needed on dust overload in nonrodent species and on the normal role of the AM in dust removal from the human lungs.

I. INTRODUCTION AND BACKGROUND

The concept of dust overloading in the lungs of Fischer 344 (F344) rats has been generally accepted by inhalation toxicologists for a number of years (Bolten *et al.*, 1983; Morrow, 1986; Lewis *et al.*, 1989). Recognized as the outcome of excessive dust exposures, particularly occurring during chronic inhalation studies, the overload phenomenon was seen initially as an experimental condition in which normally linear clearance kinetics became nonlinear (Vincent *et al.*, 1985). Subsequently, in the context of chronic toxicity testing in F334 rats, overloading became identified not only with lung burden-related protracted retention, but with other changes which seriously confounded toxicological interpretations (McClellan, 1986; Vostall, 1986). Dusts that were widely regarded as benign were found capable of producing pathologic effects, e.g., fibrosis, identical to those induced by highly toxic dusts when excessive amounts of these benign dusts were persistently retained in the lungs (Lee *et al.*, 1985).

Comparing a number of chronic inhalation studies with diverse materials, it was noted that a progressive prolongation of pulmonary dust retention apparently developed in the F344 rat when the lung burden exceeded approximately 1 mg dust per gram of lung tissue (Morrow, 1986; Morrow and Mermelstein, 1988). At higher lung burdens, that is, around 10 mg dust/g lung, pulmonary dust clearance appeared to cease almost completely. Some of the concurrent and persistent features of this progressive prolongation of pulmonary retention were histological evidence of aggregated alveolar macrophages (AM) engorged with phagocytized dust particles, evidence of a chronic inflammatory response, an increased uptake of particles in the interstitial spaces, and an increased alveolar cell hyperplasia. The subsequent development of alveolitis, granulomas, fibrosis, and pulmonary tumors increased with time and the severity of the overload condition (McClellan, 1986; Vostal, 1986; Lee *et al.*, 1985; Morrow and Mermelstein, 1988).

At a recent symposium devoted to overload-related phenomena, Witschi (1990) emphasized that overloading of a biological system was not uniquely a condition affecting the lungs; rather in toxicology generally, it has been well established that the ability of many systems to function can be overwhelmed by excessive levels of administration or exposure of a material.

Quantitation of the condition of dust overloading has been usually accomplished by serial pulmonary retention measurements of the dust and determination of the degree of pulmonary clearance suppression. Assessment of the magnitude of particle sequestration might also provide a useful criterion, for example, by the use of extensive bronchoalveolar lavaging with the unlavagable dust fraction being equated to the sequestered material. Conceivably, the measurement of other responses could also be utilized to measure the extent of overloading.

A. A Mechanistic Hypothesis for Dust Overload

A hypothesis was developed concurrently (Morrow, 1988) that the excessive levels of dust in the lungs lead to excessive engulfment of particles by AMs and after a certain degree of loading occurred, the macrophages became progressively immobilized and aggregated. Because the onset of this loss

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of AM mobility to translocate from the lungs occurred at a relatively constant lung burden for a variety of materials, the condition of overload seemed to have a generic quality.

It was further hypothesized that the collective volume, not the mass, of the phagocytized particles determined the extent of overloading (Morrow and Mermelstein, 1988; Morrow, 1988; Yu *et al.*, 1989). Since particle size is usually described in micrometers (μm), the volumetric hypothesis can be reexpressed in μm^3/AM, assuming the size of the activated AM pool in F344 rat is relatively constant, e.g., $\sim 2.5 \times 10^7$ AM per lung. Converting 1000 μg of unit density dust to an equivalent volume gives 1000 nl or 1×10^9 μm^3. If approximately 1500 μg of dust (1000 μg/g lung) is associated with the onset of overload, then 1.5×10^9 μm^3 of dust can be imagined as distributed equally among the 2.5×10^7 AM pool. This yields 60 μm^3/AM as an average volumetric load. For the virtual cessation of AM-mediated particle clearance, a 15,000 μg/g burden becomes 600 μm^3/AM (Morrow, 1988). Lehnert *et al.* (1989) have shown that the actual distribution of particles/AM is very nonuniform. Consequently, the description of average particle load per cell is an artificial, but convenient index of the overload condition in the F344 rat.

B. Possible Cell–Particle Interactions in Overload

Other presumed aspects of dust overloading are schematically portrayed in Fig. 1. Some of the complex interrelationships of macrophage activation, inflammation, and cytokine and mediator release are simplified and depicted as sequelae of an excessive dust burden and AM immobilization. To a lesser degree quantitatively and temporally, the same events and factors probably occur with all particle–AM interactions and they are presumably accentuated by particle surface properties, the amount of dust phagocytized, the intrinsic cytotoxicity of the dust, and the persistence of dust laden cells in the lung milieu (Lehnert *et al.*, 1989; Privalova *et al.*, 1980; Holt, 1990; Oberdörster, 1988). The relative or complete loss of macrophage mobility increases the likelihood of direct particle–epithelial cell interactions and interstitial localization of dust particles. Both the aggregated AM and the alveolar interstitium appear to serve as dust sequestration sites (Adamson and Bowden, 1981; White and Bhazwan, 1981).

C. Major Informational Gaps Relative to Overload

At the same symposium on overload-related phenomena cited earlier, Mauderly and co-workers (1990) and Witschi (1990) raised questions regarding the effects of lung overload on lung tumorigenesis. How important was the persistent alveolar cell hyperplasia? Did overloading produce a potential amplification of carcinogenic responses? Other issues raised included ascertaining if a true threshold existed for the overload condition (Witschi, 1990), the significance of aggregated AM as a particle sequestration compartment (Mauderly *et al.*, 1990), comparability of clearance kinetics between toxic particles and overloaded "innocuous" particles (Warheit *et al.*, 1990), mechanisms of translocation of particles through the alveolar epithelium (Warheit *et al.*, 1990), specific associations of dust overloading with factors released by the

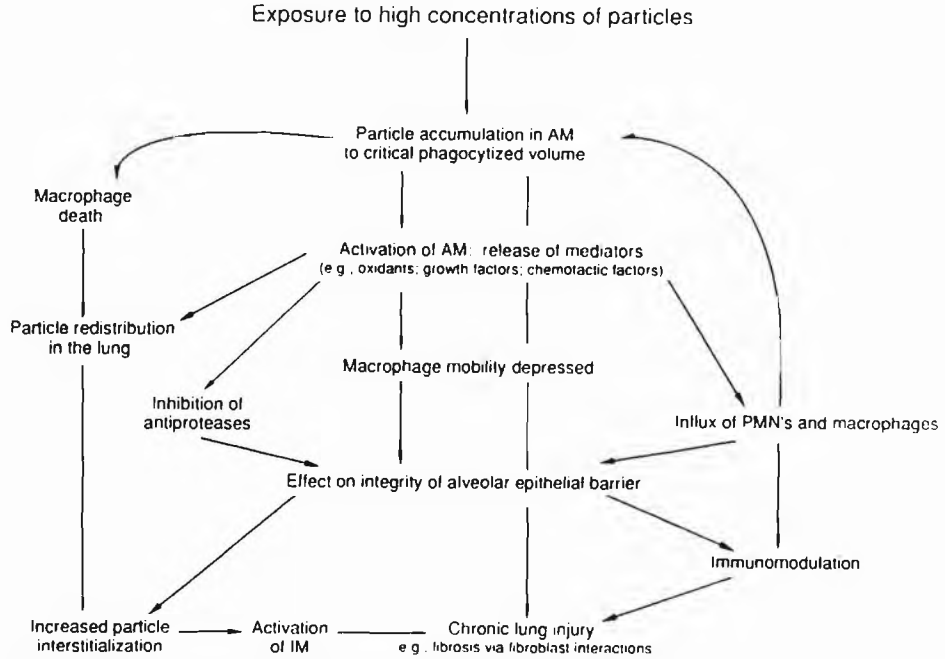

FIG. 1. Cytologic and biochemical interactions related to dust overloading. This schematic depicts some of the interactive phenomena that appear to be associated with excessive dust levels in the lungs. The arrows are not necessarily intended to indicate causes and effects, but rather associated events.

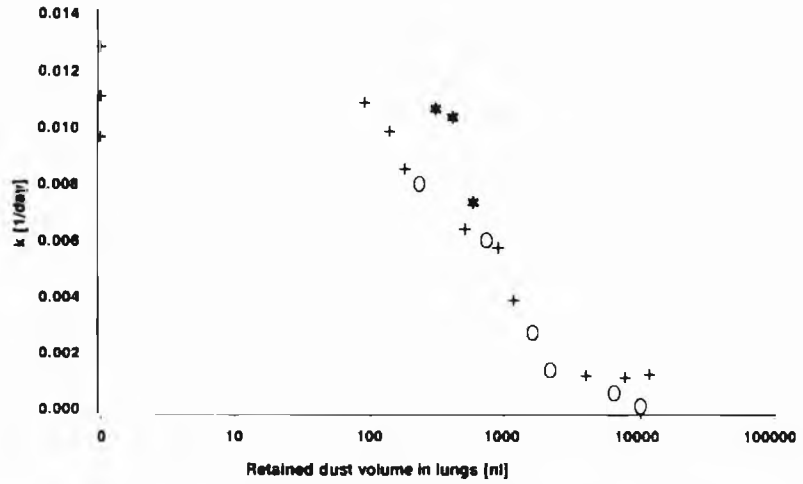

FIG. 2. Relationship between volumetric loading of lungs and dust clearance rates. This graph presents data from rat studies, viz., (+) chronic toner, (*) chronic titanium dioxide, and (0) subchronic toner, where the total lung dust burdens measured are expressed in nanoliters equivalent volume and the pulmonary dust clearance rates (k) are expressed in fractions per day. The clearance rates measured in unexposed rats (0 nl) are seen to lie between ~0.010 and 0.013 per day. All clearance rates were based on measurements of a tracer aerosol viz. ^{85}Sr-PS, except for the subchronic toner study which was based on toner measurements, per se. All data were pooled from male and female F344 rats.

AM (Driscoll *et al.*, 1990), the relevance of dust overload to other laboratory animals (Muhle *et al.*, 1990), the intraspecies variability in pulmonary particle clearance (Kreyling, 1990), the generic quality of overload as examined with different dusts (Muhle *et al.*, 1990), the kinetic modeling of dust overloading (Stöber *et al.*, 1990), and the relevance of dust overloading to man (Lippmann and Timbrell, 1990; McClellan, 1990). This summary of questions, informational deficiencies, and research needs is incomplete, but it does convey a general picture of our current concerns and limited understanding of dust overloading where unknowns greatly outnumber knowns. While this paper will not remedy this condition, it will attempt to describe relevant new data and to discuss many of these major problem areas.

II. NEW EXPERIMENTAL FINDINGS

A. Effects of Overload on Pulmonary Retention of Particles

The weight of evidence for both producing and interpreting dust overloading has come from a variety of subchronic and chronic inhalation studies and not from systematic investigations designed to better understand underlying mechanisms. However, one recent study by Oberdörster *et al.* (1991) was designed to test the volumetric basis for cessation of AM-mediated particle clearance. Here the previously described overload criteria were obviated by directly testing very low lung burdens ($\leq$100 μg) of radiolabeled 10.3 μm diameter microspheres, each of which had a volume of approximately 600 μm^3. Although promptly and completely phagocytized by the AM in the F344 rat lung, these particles were not cleared as were smaller 3.3 μm diameter microspheres, i.e., with a 60- to 70-day halftime, but were persistently retained with a 1000-day halftime. Collectively, this finding and the earlier observation of Snipes and Clem (1981) that 9 and 15 μm diameter microspheres were cleared with a ~580-day halftime and an essentially infinite halftime, respectively, indicate that the particle volume per cell "index" of overload is, in such a special circumstance, more than a mere overload indicator and close to the volumetric reality of affecting AM immobilization. Directly testing the 60-μm^3 level as the onset condition for overloading is far more difficult to undertake since there is no control of the number of particles phagocytized by each AM as there was in the 10.3-μm study where the instilled particle to AM ratio was less than 1:200 and the AMs contained either no or one particle (Oberdörster *et al.*, 1991a).

Many chronic study findings have suggested that a reduction in AM-mediated pulmonary clearance is an early event of overloading (Morrow, 1988; Yu *et al.*, 1989). Recently, a chronic inhalation study by Bellmann *et al.* (1991) with particles of copolymer plastic (toner) was conducted utilizing periodic brief exposures of F344 rats to a radiolabeled iron oxide aerosol which previously had been determined to be cleared from the lungs almost exclusively by AM (Lehnert and Morrow, 1985). It was subsequently found that the prolongation of iron oxide retention paralleled the increase in toner lung burden and the development of prolonged toner retention, thereby, implicating directly AM-mediated particle transport as the probable basis for the overload condition.

The periodic use of a radioactive aerosol, e.g., $^{59}Fe_2O_3$ or radiolabeled plastic microspheres, to measure the state of

pulmonary particle clearance raises some intriguing issues. Consider the observations of Bellmann *et al.* (1991; Fig. 2) that during the 2-year toner exposure period, the rat lungs were found, after termination and toner recovery, to contain 200 nl (2×10^8 μm^3 or ~ 8 μm^3/AM) toner and to have a pulmonary clearance rate (λ) of 0.008 per day. Concurrently, rat lungs with the same toner burden, given a brief, microgram exposure to ^{85}Sr-labeled 3.5-μm polystyrene microspheres (PS), manifest the same λ value for the ^{85}Sr-PS clearance as for toner. This λ_{PS} value was about one-half the rate of alveolar ^{85}Sr-PS clearance observed in air-exposed controls (Bellmann *et al.*, 1991). Using rat lungs from a different exposure level and/or from rats at later times, when the toner burden was $\sim$900 nl, the respective toner and ^{85}Sr-PS clearance rate constants were both about 0.006 per day. Both λs further decreased to about 0.001 per day when the toner burden became $\sim$8000 nl/lung (Bellmann *et al.*, 1991). In each case, the brief new dust exposure of ^{85}Sr-PS exhibited the same reduction of clearance rate as did the chronically administered toner. In these toner studies, titanium dioxide (TiO_2) was used as a negative control dust. When TiO_2 levels in the lungs entered the overload range, a comparable reduction in ^{85}Sr-PS clearance rates also occurred (Fig. 2). These close associations imply that the newly arrived dust intake (^{85}Sr-PS) was distributed to the same AM pool and phagocytized accordingly. Clearly, none of the overload studies cited earlier has specifically indicated that the overloaded AM pool cannot continue to phagocytize newly inhaled particles or reengulf particles from decaying resident macrophages. However, the virtually identical behavior of the new and old particle clearance, based on what must have been a nonidentical pattern of pulmonary deposition and AM engulfment of the new and old particles, is both important and remarkable in inferring, *inter alia*, that phagocytosis by immobilized macrophages must continue to occur.

B. Possible Mechanisms for Producing Generalized Overload Effects

One possible explanation for this generalized behavior of overloaded AM just described arises from the interactive phenomena depicted in Fig. 1. If we accept that the extent of dust loading in the lungs can be considered a "dose" and the amount of mediator, cytokine release, and/or inflammation (e.g., PMN infiltration) are dose-related responses, then the proposition that all AM's in the AM pool are similarly affected by a given level of "response" supplants the initial, specific, volumetric load-induced response. In other words, the induction of the overload condition brings about self-perpetuating conditions of alveolar macrophage immobilization and the concurrent development of a persistent state of inflammation, lipidosis, and immunomodulation due to persistent and possibly excessive release of growth factors, chemotactic factors, enzymes, proteases, and/or oxidants.

The credibility of this type of explanation is supported by a relationship between the reduction in dust clearance rate and the amount of PMN's in the bronchoalveolar lavage of toner-exposed rats reported by Muhle *et al.* (1991). Also Henderson *et al.* (1988) showed by serial bronchoalveolar lavage that, in F344 rats exposed to either 3.5 or 7 mg diesel soot/m^3 for up to 24 months, a dose-dependent increase in inflammatory cells, cytoplasmic and lysosomal enzymes, and protein occurred in the lavage fluid (BALF). After approximately 1 year of exposure, pulmonary fibrosis began to develop in the rat lungs and concurrently glutathione levels in BALF began to increase in relation to both the pulmonary dust burden and the degree of fibrosis. None of these effects can presently be accepted as causal; rather they must be regarded collectively as a coherent association that focuses our attention to a persistent and excessive elaboration of factors which, *inter alia*, might affect AM mobility and induce various sequelae.

A recent study by Sibille and co-workers (1989) showed that human AM not only release PMN chemotactic factors, but an apparent neutrophil inhibiting factor. This study suggests there are other modulating factors which could differentially affect AM functions, e.g., mobility. Substances presumably enhancing AM mobility include various chemotaxins (Dauber and Daniele, 1980; Adamson and Bowden, 1982); however, this evidence is based on more efficient *in vitro* encounters between AM and particles and not directly on AM mobility as regards particulate transport from the lungs per se. As already noted (Section IIA), new studies of overloaded lungs strongly suggest phagocytic motility and AM migratory ability are differentially affected: hence these forms of mobility are not completely interdependent. Substances reducing AM mobility are even less well identified and understood, but prominent candidates in this regard are such factors as macrophage inhibition factor and phospholipids, both elaborated by activated AMs. Phospholipidosis, experimentally induced by chlorphentermine in the F344 rat, is accompanied by aggregated AMs and a reduction in particle clearance (Ferin, 1982). Lipoproteinaceous lavage fluid from human lungs has also been demonstrated to impair phagocytic function, but not intracellular degradative activity (Nugent and Pesanti, 1983). It is interesting to note that McNulty and Reasor (1981) examined phospholipodotic AM from rats treated with chlorphentermine and found both the *in vitro* phagocytic and bactericidal activities were increased relative to AM from untreated rats. The recent work on the over production of surfactant induced by a variety of particulate materials appears germane to this general topic (Miller and Hook, 1990), as is the study of Khan *et al.* (1990) on AM–particle binding by components of the alveolar lining layer. For example, surfactant apoprotein A binds to AM and acts a chemotactic factor (Beinenstock and Gauldie, 1988) that could, when increased, possibly reverse the normal chemotactic and chemokinetic gradient that affects AM mi-

gration. Despite the attractiveness of the foregoing studies in providing mechanistic information on overload, most have not investigated overloaded animals.

Driscoll and co-workers (1990) recently reported that when 10 mg or more of TiO_2 ($\geq$12,500 nl) was instilled into the lungs of ~200-g F344 rats, there was subsequently a transient increase in tumor necrosis factor (TNF) release and a sustained increase in fibronectin release from AM obtained by bronchoalveolar lavage. In contrast, the use of a simple *in vitro* system of TiO_2 dust and AM did not result in the release of either factor. Hence the pulmonary environment of the AM was essential for this AM activation. This conclusion agrees with the views of Beinenstock and Gauldie (1988) that pulmonary cellular interactions with the AM are important for the development of chronic lung inflammation and immunity.

Lehnert *et al.* (1990) attempted to evaluate AM migratory behavior as a function of dust burdens below and within the range of dust burdens producing overload. Intratracheal administrations of 86, 1000, or 3700 μg of 2.13 μm diameter polystyrene microspheres (p = 1.23 g cm^{-3}) were made in groups of rats and the respective lung retentions followed. On post instillation Days 14, 57, and 85, lavaged AM were assessed, *in vitro*, for their abilities to migrate through a 5-μm pore membrane in the presence or absence of activated rat serum. Compared to AMs without particles, stimulated AMs, i.e., those with activated serum, from all dose levels exhibited reduced migratory behavior at 14 and 57 days but exhibited increased migratory behavior at 85 days. In general, a preferential migration was found with particle-free AMs and AMs having the smaller particle burdens.

In a further effort to examine the importance of overloaded AM in explaining reduced pulmonary particle clearance, Lehnert *et al.* (1990) examined lavaged AM from rats receiving intratracheally ~3.7 mg (3000 nl) of plastic microspheres (6.8×10^8 spheres of 2.13 μm diam) and found that the number of AM containing an engulfed particulate volume equal to or greater than 500 nl was sufficient to explain the prolongation of retention in distinction from the shorter retention times obtained with rats administered lesser particulate burdens ~70 and ~810 nl per lung.

C. *Reversibility of the Overload Condition*

A critical consideration pertinent to the foregoing evidence and the overload condition generally is the matter of reversibility. Resolution of the overload condition might be expected to vary in relation to the extent of its development. As long as some AM-mediated particle clearance continues and there exists the concurrent probability of a redistribution of particles due to release and reengulfment by decaying and recruited AMs, respectively, a progressive reduction in dust loading would be expected to occur. In contrast, if the AM-mediated clearance is effectively arrested, then little or no resolution of the overload condition in the AMs might be expected, while the concurrent induction of widespread lung pathology, e.g., progressive granulomatous changes, would be expected to exacerbate the problem by hindering dust clearance by other means than overload (Bohning *et al.*, 1982).

One experimental investigation of reversibility from a condition of dust overload was undertaken by the Fraunhofer group (Creutzenberg *et al.*, 1989; Bellmann *et al.*, 1989). Following a 90-day subchronic exposure to a test toner at 0, 10, and 40 mg m^{-3}, female F344 rats were serially euthanized in groups at 3-month intervals over an additional 15-month post exposure period at which time bronchoalveolar lavages and histopathologic evaluations were performed and toner retention was directly measured. Concurrently, tracer amounts of a ^{85}Sr-labeled polystyrene aerosol (3.5 μm diam) were inhaled by eight animals per group and retention measurements were made for a subsequent 90 days by thoracic gamma counters. The mean maximal lung toner burdens achieved from the 3-month exposure were 0.4 and 3.0 mg per lung for the 10 and 40 mg m^{-3} concentrations, respectively. For the 0.4 and 3.0 mg/lung rats, respectively, 70% of the 0.4-mg and only 12% of the 3.0-mg toner burdens were cleared during the 15-month post exposure period.

Although the correspondence between the toner retention and the tracer polystyrene retention seen in the Fig. 2 subchronic data was not quantitatively the same as found in the "recovery" study, as far as AM-mediated particle removal was concerned, there seemed to be evidence of complete reversibility in the low exposure group as the rats soon again cleared ^{85}Sr-PS at the rate of air controls. With the high exposure group, there was no clear evidence of recovery of the suppressed pulmonary clearance. Using a compartmental model developed for this study which included immobilized macrophages as a novel sequestration compartment, one having a chemotactic effect on other macrophages, Bellmann *et al.* (1990) deduced that some recovery occurred, i.e., a shift in particles from the immobilized macrophage compartment to the mobile macrophage compartment occurred at the rate of ~0.06% per day. On this basis, only a partial resolution of the overload condition could have occurred during the animal's lifetime. Similar conclusions were drawn regarding the reversibility of biochemical and cytologic changes found in bronchoalveolar lavagate (Creutzenberg *et al.*, 1989), viz., the more advanced the condition of dust overload, as judged by lung burden and particle clearance reduction, the slower and more incomplete the return to normal values.

D. *Relevance of Dust Overloading and AM-Mediated Clearance to Other Species*

The available evidence indicates that AM-mediated particle clearance is the central functional feature of overloading with relatively insoluble dusts in the Fisher 344 rat. To the extent that other dust clearance processes operate concur-

rently, e.g., solubilization, the effect of a given dust loading on alveolar retention could conceivably be either dominant or comparatively small. The relative importance of AM-mediated particle clearance among different species compared to clearance by solubilization thereby becomes an important issue since there is evidence that different species rely on a different combination of clearance processes (Snipes *et al.*, 1989). To date, a very limited effort to determine if dust overloading occurs in other species has been manned. Muhle *et al.* (1990) recently reviewed several studies which indicate a similar condition of dust overloading by insoluble particles does occur in the Syrian hamster and the mouse with findings that are qualitatively comparable in terms of pulmonary clearance suppression and histopathologic changes. Since the findings are limited and far more variable than in the rat, more data are needed to establish dust burden comparability within a factor of two.

From another perspective, i.e., species distinctions in AM-mediated clearance, a recent balance study in sheep by Langenback and co-workers (1990) demonstrated that >99% of the ^{57}Co-labeled carbonized polystyrene particles (2.85 μm CMD) cleared from the lungs was recovered in the feces. The overall lung retention curve was described as a triphasic first order process: $R = 0.303 \exp(-2.36t) + 0.439 \exp(-0.174t) + 0.259 \exp(-0.023t)$, where t is expressed in days. The first phase (0.3-day halftime) was interpreted to be tracheobronchial, the second phase was considered kinetically transitional, and the third slower clearance phase was deemed alveolar. This inhalation study with three sheep involved numerical particle depositions of $\sim 2.5 \times 10^8$ particles per sheep lung. Estimating the volumetric median particle diameter as 3.27 μm, each particle had a 18.8-μm^3 volume. Assuming the AM pool size was equivalent to their reported average lavagable AM value, then ~ 1.2 μm^3/AM can be assumed as the mean particulate volume per cell burden in this 100-day post exposure study. An overload condition was obviously not present. Although Langenback *et al.* (1990) suggested the sheep lung should be considered a model for the human lung in studies of particle retention, this study in fact, indicates that AM-mediated clearance in the sheep ($t_{\frac{1}{2}}$ ~30 day) is even more rapid than in the rat and according to other species comparisons, the rat has a more rapid alveolar clearance than guinea pigs, dogs, monkeys, and humans (Snipes *et al.*, 1989).

E. Pulmonary Dust Retention in Man

In relation to species distinctions, it is useful to review several recent studies of dust retention in man following brief exposures to insoluble particles. Three studies, in particular, typify the kinds of information that are being obtained currently and how they are being interpreted. First, the study of Stahlhofen *et al.* (1981) using radioactively labeled 4.7 μm diameter Teflon particles concluded that tracheobronchial clearance was complete within 48 hr and that the rate of "mechanical" (AM-mediated) clearance was about 0.006 day^{-1}, indicating a 115-day pulmonary halftime. For technical reasons, radioactive measurements were made for a maximum of 14 days. Radioisotopic leaching (which was minimal) was taken into account by this halftime value.

Bailey *et al.* (1985), in an acute inhalation study designed to differentiate clearance by dissolution from clearance by AMs, used radioactive fused aluminum silicate particles and reported AM-mediated transport started with a rate of 0.004 day^{-1}, fell to 0.001 day^{-1} at 200-days post exposure, and continued to decrease thereafter. These data infer that AM-mediate transport in the human lungs is nonlinear, but can be described with apparent first-order halftimes ranging from 174 to >700 days. These interpretations were based on the use of a constant particle dissolution rate of 3.3×10^{-8} g cm^2 day^{-1} determined *in vitro*.

The third human study, by Foster *et al.* (1989), was designed to be a balance study wherein an accountability of the test dust ($^{57}Co_3O_4$) was maintained by the combined use of external counting of lung burden and urine and fecal collections over the 230-day study. After a few days of tracheobronchial clearance, the investigators reported that the measured pulmonary retention halftimes in their subjects ranged between 150 and 250 days. Taking into account the urinary and fecal excretion rates, which they associated with dissolution and AM-mediated (mechanical) clearances, respectively, they concluded that about $\frac{3}{4}$ of the pulmonary clearance was due to dissolution.

Using the clearance rate relationship

$$\frac{1}{\lambda_{eff}} = \frac{1}{\lambda_{diss}} + \frac{1}{\lambda_{AM}},$$

their data provide $\frac{1}{\lambda_{eff}} = \frac{1}{0.0043\ day^{-1}} + \frac{1}{0.0010\ day^{-1}}$,

which yields an effective retention halftime of 172 days in conformity to the measured data. This mean effective halftime can be seen to be derived from a 231-day halftime for dissolution and a 640-day halftime for AM-mediated clearance.

F. Relevant Retention Studies Not Involving Dust Overload

It is important to scrutinize inhalation study findings that do not involve overloading and to verify the absence of effects deemed attributable to overloading by benign dusts. An excellent example is the study of talc deposition at three different exposure levels in F344 rats and in mice by Pickrell *et al.* (1989). The talc lung burdens in rats after 20-day exposures (6 hr/day, 5 day/wk) ranged from 70 to 720 μg (22–229 nl) talc per gram lung and when these burdens were normalized for the exposure concentrations of 2.3, 4.3, and 17 mg/m^3, the normalized dust burdens were approximately

10, 12, and 13 nl talc/g lung/mg/m^3, respectively. For mice, with lung burdens ranging between 100 and 1020 μg/g lung (32–324 nl/g), the normalized lung burden for 2.2, 5.7, and 20.4 $\mu g/m^3$ exposure levels was 15, 16, and 16 nl talc/g lung/mg/m^3, respectively. At these low lung burdens of talc in both species, overloading would not be predicted to occur. These data indicate a proportionate lung burden exposure concentration ratio, which would not be expected to occur if the pulmonary clearance rate was not relatively constant for each species, i.e., independent of lung burden. The presence of first-order clearance kinetics and the absence of abnormal histopathologic findings in this study also convey a general picture of a multiple exposure study to a relatively benign dust not involving any characteristics of the condition of dust overloading.

In another study Hemenway *et al.* (1990) examined several silica aerosols of differing fibrogenicity, viz., cristobolite which is highly fibrogenic and MIN5 quartz and TAFQ quartz, both of which possess a weak fibrogenic potential. These investigators found at levels of SiO_2 below 500 nl/g lung, i.e., below the overload range, that the rate of cristobolite clearance was substantially slower than that measured for comparable lung burdens of the less toxic quartz forms in male F344 rats.

Studies involving ultrafine particles (<0.01 μm diameter) at lung burdens of about 100 nl of dust (Oberdörster *et al.*, 1992; Ferin *et al.*, 1991) have demonstrated significant interstitial uptake. Epithelial permeation by ultrafine particles appeared to be a competitive phenomenon with AM engulfment, hence, superficially resembled the effect of decreased AM phagocytic and clearance function accompanying dust overload. The ultrafine particles also appeared to induce an inflammatory response disproportionate to comparable lung dust burdens of the more usual 0.1–10 μm diameter particles.

A number of technically different studies have been reported which indicate that particle retention under certain conditions is prolonged in the tracheobronchial tree (e.g., Patrick and Sterling, 1977 and Stahlhofen, 1989). These reported observations are no more germane to the condition of dust overloading than are numerous studies of the long-term pulmonary retention normally found with low lung dust burdens; consequently, such studies have not been included in this review. Exceptions are the several studies, just described, which provide information quantitatively differentiating overload from nonoverload conditions.

III. DISCUSSION

The 60 μm^3/AM value is convenient to use as though it were a mean volumetric threshold for overloading, but, in fact, extant data do not support a threshold, as such, but indicate a range of values from about 400 to 1500 nl/g lung for the F344 rat or 25 to 90 μm^3/AM. Although less uncertainty applies to the 600 μm^3/AM value for the volumetric load causing a cessation of AM-mediated particle clearance, it, too, must be considered to represent a range of values. Part of the difficulty in establishing these values more precisely, apart from biological variation, is due to the variability in measuring the response criterion, prolongation of retention. It may be reasonable to assume that at least a doubling of the "normal" retention halftime is necessary in the F344 rat to establish that a statistically significant prolongation of retention has occurred. For determining the cessation of clearance, one often finds it difficult to differentiate with confidence, for example, between a 2000-day halftime and an infinite halftime. In terms of the life span of the F344 rat, the difference, dosimetrically, in a chronic exposure study is trivial. Consequently the "virtual" cessation of pulmonary clearance is judgmental and one could arbitrarily accept a greater than 10-fold increase in retention halftime as a suitable criterion.

Correlating lung dust overload with criteria other than prolongation of pulmonary retention should be practical, but suitable dose–effect data are presently lacking. Pathologic criteria, e.g., fibrosis, depend on temporal considerations which have been shown to be valid dosimetrically in the F344 rat in relating toner dose (e.g., $\mu g \cdot$ days/g lung) to the *incidence* of pulmonary fibrosis (Muhle *et al.*, 1991). However, this dosimetry does not necessarily serve as a generic basis for indicating either the presence or the extent of dust overloading per se unless one can demonstrate the absence of intrinsic fibrogenicity, as was the case with toner. A refinement of the dosimetric approach (Mermelstein *et al.*, 1991) suggests the possibility of characterizing relative bengin dusts, e.g., toner, on the basis of fibrosis induction requiring >500 mg days dust/g lung under chronic exposure conditions. A simple calculation reveals that an overload condition must be achieved in the lungs of F344 rat to reach or exceed this cumulative dose.

Another possible approach for the assessment of dust overloading is based on determining the sequestered or nonlavagable pulmonary dust. For example, after extensive bronchoalveolar lavaging, Oberdörster *et al.* (1991) treated the nonlavagable dust in the lung as equivalent to the sequestered dust. These investigators presumed that this nonlavagable fraction consisted of dust in both AM aggregates and the interstitial spaces and thereby constituted the dust not subject to AM-mediated clearance. This fraction increased as the lung burden of dust increased and the pulmonary clearance rate of the dust decreased.

Incorporation of dust into the *interstitium* has been shown to increase as the number of particles acutely administered to the lungs increases (Adamson and Bowden, 1981). Analogous conditions arise from increasing inhalation exposure concentrations which increase the rates of deposition. Also, when the ability of the AM to function as a mobile phagocytic cell is compromised, as with a cytotoxic dust, pulmonary

particle retention is prolonged. This was amply demonstrated in the study of cristobolite by Hemenway *et al.* (1990) cited earlier. Recent studies with ultrafine particles (~20 nm diameter) suggest that phagocytic uptake may not be as efficient for ultrafine particles as with larger particles and that increased interstitial penetration by ultrafine particles occurs without excessive dust burdens (Oberdörster *et al.*, 1992; Ferin *et al.*, 1991). Additional studies are needed to determine if the toxic sequalae induced by ultrafine TiO_2 particles are qualitatively the same as seen with large TiO_2 particles (MMAD ~1 μm) under conditions of overload.

Using dust build-up in the tracheobronchial lymph nodes as an index of interstitial dust uptake, the work with ultrafine particles provides a coherent picture with the size of the sequestered particle depot and lymph nodal uptake observed in the diesel and carbon black studies that involved dust overload (Strom *et al.*, 1988, 1989; Yu *et al.*, 1989). The inhalation study of Greenspan and co-workers (1988) that evaluated AM-mediated particle clearance of TiO_2 particles with and without the concurrent inhalation of an aerosol of cadmium chloride, which is cytotoxic to the AM, also found lymph node build-up was greater when AM function was depressed.

Several investigators have emphasized the importance of evaluating the sequestered dust "compartment" in order to properly describe the deposition and clearance kinetics seen in normal and overload conditions (Strom *et al.*, 1989; Stöber *et al.*, 1989; Smith, 1985). Some of the kinetic models developed include both reversible and irreversible sequestration processes and although quantitative experimental data are not available to document all these features of dust sequestration, the models are intuitively sound and quantitatively linked to lung burden and macrophage clearance, including lymph nodal uptake, but remarkably important informational gaps exist on basic cytologic parameter in man, viz., AM turnover rates. It is difficult to obtain a coherent picture of AM-mediated clearance (alias mechanical clearance) from recent human studies. The study by Stahlhofen *et al.* (1981) seems very straightforward since there was no clearance by dissolution and no leaching correction was needed, but the actual retention measurements were limited to only 14 days post exposure. In two cases cited (Bailey *et al.*, 1985; Foster *et al.*, 1989), measurements of the leaching and dissolution rates *in vitro* were equated to the pulmonary dust dissolution rate in the lungs. These rates, in turn, were applied to the overall or effective pulmonary clearance rates which were directly measured, and the AM-mediated clearance was determined inferentially. The study by Bailey *et al.* (1985) indirectly determined the AM-mediated clearance and found that it appeared to be time dependent. The range of halftime values used to describe the overall retention curve fits the halftime reported by Stahlhofen *et al.* (1981) at early times and at later times agrees with the halftime reported by Foster *et al.* (1989). In the Foster *et al.* (1989) study, the urinary excretion rate was coupled to the pulmonary dust dissolution rate, the fecal elimination rate was used to estimate the AM-mediated clearance rate, and the combination of the two rates was found to be compatible with the overall clearance rate, which was measured directly (Foster *et al.*, 1989). The excellent design of the Foster *et al.* (1989) study makes their findings particularly persuasive. An important point of contention remains, however, in their relating urinary excretion to pulmonary dissolution.

In the absence of rational explanations as to why AM-mediated clearance in the human lungs should appear to vary so much or become slower with time, one is tempted to conclude that the disparate findings in the human studies are a consequence of technical and interpretive problems. Obviously, mechanistic information on human pulmonary clearance is critically needed. Now that the use of bronchoalveolar lavage has become almost routine in humans, exciting data on particulate disposition and AM-mediated clearance should be forthcoming.

Since clearance by dissolution and by AM-mediated clearance are "parallel," competitive pulmonary clearance processes, one can readily comprehend that the greatest distinction possible in the pulmonary clearance retardation by overloading must occur in the absence of any clearance by dissolution. For illustrative purposes, the 115-day halftime of Stahlhofen *et al.* (1981) could result in a range of clearance rates from 0.0060 day^{-1}, with normal AM clearance, to 0.0000 day^{-1} with no AM clearance, so order-of-magnitude effects of overloading would be easily demonstrable. If a dissolution rate of 0.0006 day^{-1} was typical of the least soluble materials in the lungs and was manifest with or without overload, then a factor of ~10 would distinguish the full presence or complete absence of AM-mediated clearance in the lungs, for these materials, i.e., 105- versus 1155-day halftimes [ln 2/(0.006 day^{-1} + 0.0006 day^{-1}) vs ln 2/0.0006 day^{-1}], still an important, resolvable prolongation in retention. However, if the 640- to 700-day halftime values of Foster *et al.* (1989) and Bailey *et al.* (1985) are used to set a normal rate of human AM-mediated clearance at 0.0010 day^{-1}, then its full presence or complete absence results in only a factor 2.7 prolongation in halftime, barely greater than the factor 2 operational definition of a significant change in retention due to overloading in the rat. For example, with a particulate material such as the $^{57}Co_3O_4$ used by Foster *et al.* (1989), having a reported dissolution rate of 0.0043 day^{-1}, it can be deduced that a 231-day halftime would be associated with a complete absence of AM-mediated clearance and a 131-day halftime would be expected with a fully normal AM-mediated clearance; this is approximately the range of halftimes observed experimentally, although only tracer quantities of dust were utilized and a condition of overload should not have occurred.

These foregoing examples underscore the difficulty of resolving both the importance and the rate of AM-mediated

particle clearance in human subjects given the problems of biological variability, possible temporal effects, and technical limitations, while not even considering such problems as the cytotoxic effects of materials, the AM cell turnover (Roy, 1989), the possibility of tissue binding of the dissolved particulate phase (Oberdörster *et al.*, 1979), and the uncertain generic suitability of *in vitro* (Morrow *et al.*, 1968) or cell culture (Kreyling *et al.*, 1990) measurements of dissolution rates for pulmonary clearance.

Another major feature of dust overloading stems from the critical functions AMs have in both host defense and lung injury. Several investigators (Brain, 1980; Herscowitz, 1985; Bowden, 1987) have described the apparently paradoxical role of AMs in these regards. Suffice it to say, there are so many possibilities that almost any postulated causality can be supported. That we have too many explanations available as to how immobilized macrophages could elaborate self perpetuating factors is complicating, but at the same time, the probability of such an effect is considerable.

Examples of the factors elaborated by the AM which could lead to particle interstitialization, prolonged inflammatory changes, and lung injury include growth factors for fibroblasts (Bitterman *et al.*, 1982); hydrogen peroxide (Fisher and Bostick-Bruton, 1982) and hydroxyl radical release (Ward *et al.*, 1983); neutrophil chemotactic factors (Hunninghake *et al.*, 1978); enzymes, e.g., lipoprotein lipase (Okabe *et al.*, 1984); mediators for lymphoproliferative responses (Laughter *et al.*, 1977); immunomodulating factors, e.g., IL-1, interferon (Acton and Myrvik, 1966), and prostaglandins (Hseuh and Kuhn, 1979); and various mediators of host defense, e.g., complement components (Brain, 1980) and leukotrienes (Hseuh and Sun, 1982). The important experimental findings of Driscoll *et al.* (1990) on TNF and fibronectin described earlier, point up the difficulties of examining these various factors in simple *in vitro* systems and the importance of the intact pulmonary milieu for determining or modulating their interactive effects.

Thus, we can presently visualize the condition of dust overloading as being initiated by an excessive dust exposure which, in turn, leads to increased phagocytic uptake of particles eventually reducing AM mobility, as judged by reduced AM-mediated particle clearance, even in the absence of an intrinsic cytotoxic action by the dust. This general state of AM immobilization represents a spectrum of suppressed mobilities, which we can presently describe by an average phagocytized volume (or mass). According to the magnitude of this immobilization, both a prolongation of dust clearance and a persistent elaboration of AM factors tend to increase the particle retention and pulmonary dose and to sustain the condition of overloading throughout the alveolar region. The relative importance and the pattern of these actions conceivably change with time. The persistence of a given effect is not necessarily evidence for a singular pattern of causation.

Some implications of dust overloading and its sequelae on evaluating the pulmonary toxicity of dusts and in undertaking risk assessments have been cited (Introduction and Background) and inferred throughout this paper. It is evident that our extant knowledge of the dust overload condition in the lungs of F344 rats is presently sufficient to allow us to conclude that overloading seriously confounds toxicological interpretations in that species. Differentiating overload effects from those induced by the intrinsic toxicity of the inhaled material relies to a major extent on the toxic potency of the material. If significant functional and morphologic changes are induced at lung burdens well below 1000 nl/g lung, as was the case in the study of Hemenway *et al.* (1990), the most plausible explanation for these changes is the intrinsic toxicity of the material. Obviously the problem of differentiation becomes increasingly difficult, the lower the intrinsic toxicity of the material, i.e., the closer the dust resembles dusts in the former "nuisance" dust or present particles not otherwise classified categories used for occupational threshold limit values by the American Conference of Governmental Industrial Hygienists (Morrow *et al.*, 1991).

If the condition of overloading is only sufficient to prolong pulmonary dust retention significantly without producing *important* cytologic or morphologic changes, it probably will have little impact on the experimental findings and interpretations since this is basically a matter of increasing pulmonary dose without concomitantly increasing evidence of adverseness. In other words, a range of doses must exist for each substance over which equivocal evidence to frank evidence of toxicity are manifest. The width of this range will doubtlessly vary with different benign materials and the evaluation criteria examined, but the general picture may be more complex. For example, if a second "benign" material or an infective agent is introduced into this minimal overload senario, would some predisposition be demonstrable due to the partial compromise of host defenses and the concurrent increase in pulmonary dose? At present, we have a limited insight into this problem from experimental studies. Recent studies by Gilmour and co-workers (1989a,b) utilized TiO_2 exposure regimes which should have produced conditions of dust overloading, which they expressed as "macrophage blockade," i.e., 10 consecutive days of exposure to 20 mg/m^3 TiO_2, 20 hr per day. The exposed CBA mice and unexposed control mice were serially challenged with an aerosol of *Pasteurelle haemolytica*. The studies showed impaired bacterial clearance in the TiO_2-exposed mice. It is unfortunate that the lung burdens of TiO_2 were not actually measured in these studies, but the conclusion expressed by the investigators, that the pulmonary immune function was compromised in proportion to the duration of exposure, is especially significant.

The potential predisposition of individuals who are smokers or who have other risk factors which might effect the onset and degree of dust overloading needs to be examined

in both humans and animal models. As Bohning *et al.* (1982) clearly revealed, smoking has very important suppressive effects on pulmonary clearance in man.

Another outgrowth of the overload studies has been the greater realization of the fact that, kinetically, many first-order descriptions of dust retention in the respiratory system have no mechanistic basis. The use of simple first-order clearance kinetics is usually appropriate to normal AM-mediated clearance, but not with clearance by dissolution, nor with AM-mediated clearance in an overloaded lung, nor with the clearance of a cytotoxic dust (Morrow, 1989). The use of first-order kinetics where no mechanistic basis exists is widespread and an acknowledged, powerful, curve-fitting method; however, the usual approaches to curve stripping (method of residuals) does not produce "unique solutions." Hence, there is a considerable likelihood of similar retention data being analyzed in dissimilar ways and producing different first-order descriptions: products which complicate an already difficult situation. The development of respiratory models which have realistic functional and cytological bases and appropriate kinetic descriptions (e.g., Yu *et al.*, 1989; Stöber *et al.*, 1990, 1989) must pertain to conditions of both reasonable and excessive dust burdens and dispense with the arbitrary use of first-order kinetics.

The foregoing discussion touches on a number of problems which presently hinder our understanding of dust overloading. Some will be solved by systematic study: some will continue to challenge our ingenuity for many years to come. At the very least, we have gained a new appreciation of the F344 rat as an experimental model for inhalation toxicology. We may find, however, that dust overloading has comparably important implications in most species, including man. If this appears to be the case, then a decade of well-funded, properly designed studies will be needed to give us an appreciation of the magnitude of the overall problem, which includes *inter alia*, much needed relevant basic information on human alveolar cytokinetics, biochemistry, and pathogenesis. The potential impact of dust overloading on toxicological testing (Lewis *et al.*, 1989) and occupational dust standards (Morrow *et al.*, 1991) is a matter in need of urgent resolution. Concurrently, as Lippman and Timbrell (1990) stated, we also need to more firmly establish which particle properties are to be measured and to verify that we can relate defined exposure parameters to health outcomes measured in human studies or extrapolated from animal studies.

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REFERENCES

Acton, J. D., and Myrvik, Q. N. (1966). Production of interferon by alveolar macrophages. *J. Bacteriol.* **91,** 2300–2304.

Adamson, I. Y. R., and Bowden, D. A. (1981). Dose response of pulmonary macrophagic system to various particulates and its relationship to transepithelial passage of free particles. *Exp. Lung Res.* **2,** 165–175.

Adamson, I. Y. R., and Bowden, D. A. (1982). Chemotactic and mitogenic components of the alveolar macrophage response to particles and neutrophil chemoattractant. *Am. J. Pathol.* **109,** 71–77.

Bailey, M. R., Fry, R. A., and James, A. C. (1985). Long-term retention of particles in the human respiratory tract. *J. Aerosol Sci.* **16,** 295–305.

Beinenstock, J., and Gauldie, J. (1988). Cellular interactions in lung inflammation and immunity. *Postgrad. Med. J.*, Suppl. 4, 111–119.

Bellmann, B., Muhle, H., Creutzenberg, O., Kilpper, R., Morrow, P. E., and Mermelstein, R. (1989). Reversibility of clearance impairment after subchronic test toner inhalation. *Exp. Pathol.* **37,** 234–238.

Bellmann, B., Muhle, H., Creutzenberg, O., and Mermelstein, R. (1990). Recovery behavior after dust overloading of lungs in rats. *J. Aerosol Sci.* **21,** 377–380.

Bellmann, B., Muhle, H., Creutzenberg, O., Dasenbrock, C., Kilpper, M. R., MacKenzie, J. C., Morrow, P. E., and Mermelstein, R. (1991). Lung clearance and retention of toner, utilizing a tracer technique during a long-term inhalation study in rats. *Fundam. Appl. Toxicol.* **17,** 300–313.

Bitterman, P. B., Rennard, S. I., and Hunninghake, G. W. (1982). Human alveolar macrophage growth factor for fibroblasts: Regulation and partial characterization. *J. Clin. Invest.* **70,** 806–822.

Bohning, D. E., Atkins, H. L., and Cohn, S. H. (1982). Long-term particle clearance in man: Normal and impaired. *Ann. Occup. Hyg.* **26,** 259–271. [Inhaled Particle V].

Bolton, R. E., Vincent, J. H., Jones, A. D., Addison, J., and Beckett, S. T. (1983). An overload hypothesis for pulmonary clearance of UICC amosite fibers inhaled by rats. *Br. J. Ind. Med.* **40,** 264–272.

Bowden, D. H. (1987). Macrophages, dust and pulmonary diseases. *Exp. Lung. Res.* **12,** 89–107.

Brain, J. D. (1980). Macrophage damage in relation to the pathogenesis of lung diseases. *Environ. Health Perspect.* **35,** 21–28.

Cole, F. S., Matthews, W. J., Rossing, T. H. *et al.* (1983). Complement biosynthesis by human bronchoalveolar macrophages. *Clin. Immunol. Immunopathol.* **27,** 153–159.

Creutzenberg, O., Muhle, H., Bellman, B. L., Kilpper, R., Morrow, P. E., and Mermelstein, R. (1989). Reversibility of biochemical alterations in bronchoalveolar lavagate upon cessation of dust exposure. *Exp. Pathol.* **37,** 243–247.

Dauber, J. H., and Daniele, R. P. (1980). Secretion of chemotaxins by guinea pig lung macrophages. I. Spectrum of inflammatory responses. *Exp. Lung Res.* **1,** 23–32.

Driscoll, K. E., Maurer, J. K., and Crosby, L. L. (1990). Overload on lung clearance is associated with activation of alveolar macrophage tumor necrosis factor and fibronectin release. *J. Aerosol Med.* **3**(Suppl. **1**), 83–92.

Ferin, J. (1982). Alveolar macrophage mediated pulmonary clearance suppressed by lung-induced phospholipidosis. *Exp. Lung Res.* **4,** 1–10.

Ferin, J., Oberdörster, G., Soderholm, S., and Gelein, R. (1991). Pulmonary tissue access of ultrafine particles. *J. Aerosol Med.* **4,** 57–68.

Fisher, R. I., and Bostick-Bruton, F. (1982). Depressed T cell proliferative responses in Hodgkin's disease: Role of monocyte-mediated suppression via prostaglandins and hydrogen peroxide. *J. Immunol.* **129,** 1770–1774.

Foster, P. P., Pearman, I., and Ramsden, D. (1989). An interspecies comparison of the lung clearance of inhaled monodisperse cobalt oxide particles. Part II. Lung clearance of inhaled cobalt oxide in man. *J. Aerosol Sci.* **20,** 189–204.

Gilmour, M. I., Taylor, F. G. R., Baskerville, A., and Wathes, C. M. (1989a). The effect of titanum dioxide inhalation on the pulmonary clearance of Pasternella haemolytica in the mouse. *Environ. Res.* **50,** 157–172.

Gilmour, M. I., Taylor, F. G. R., Baskerville, A., and Wathes, C. M. (1989b). Pulmonary clearance of Pasternella haemolytica and immune responses in mice following exposure to titanium dioxide. *Environ. Res.* **50,** 184–194.

Greenspan, B. J., Ferin, J., and Morrow, P. E. (1988). The effects of aerosol exposures to cadmium chloride on the clearance of titanium dioxide from the lungs of rats. *Exp. Lung Res.* **14,** 491–499.

Hemenway, D. R., Abscher, M. P., Trombley, L., and Vacek, P. M. (1990). Comparative clearance of quartz and cristabolite from the lung. *Am. Ind. Hyg. Assoc. J.* **51,** 363–369.

Henderson, R. F., Pickrell, J. A., Jones, R. A., Sun, J. D., Benson, J. M., Mauderly, J. J., and McClellen, R. O. (1988). Response of rodents to inhaled diluted diesel exhaust: Biochemical and cytological changes in bronchoalveolar lavage fluid and in lung tissue. *Fundam. Appl. Toxicol.* **11,** 546–567.

Herscowitz, H. B. (1985). In defense of the lung: Paradoxical role of the pulmonary alveolar macrophage. *Ann. Allergy* **55,** 634–648.

Holt, P. G. (1990). Inflammation in organic dust-induced lung disease: New approaches for research into underlying mechanisms. *Am. J. Ind. Med.* **17,** 47–54.

Hsueh, W., and Kuhn, C. S. (1979). Prostaglandin secretion in rabbit alveolar macrophages and its relationship to phagocytosis. *Chest* **75S,** 249–2515.

Hsueh, W., and Sun, F. F. (1982). Leukotriene B4 biosynthesis by alveolar macrophages. *Biochem. Biophys. Res. Commun.* **106,** 1085–1091.

Hunninghake, G. W., Gallin, J. I., and Fauci, A. S. (1978). Immunologic reactivity of the lung: The *in vivo* and *in vitro* generation of a neutrophil chemotactic factor by alveolar macrophages. *Am. Rev. Respir. Dis.* **117,** 15–23.

Khan, M. F., Gallagher, J. E., and Brady, A. R. (1990). Effect of alveolar lining material on particle binding and phagocytosis. In *Proceedings of the Seventh International Pneumoconisos Conference.* US DHHS Publ. No. 90-108, Vol. 2, p. 1488.

Kreyling, W. G. (1990). Interspecies comparison of lung clearance of "insoluble particles." *J. Aerosol Med.* **3**(Suppl. 1), 93–110.

Kreyling, W. G., Godleski, J., Kariya, S., Rose, R. M., and Brain, J. D. (1990). *In vitro* dissolution of uniform cobalt oxide particles by human and canine alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* **2,** 413–422.

Langenback, E. G., Bergofsky, E. H., Halpern, J. G., and Foster, W. M. (1990). Supramicron-size particle clearance from alveoli: Route and kinetics. *J. Appl. Physiol.* **69,** 1302–1308.

Laughter, A. H., Martin, R. R., and Twomey, J. J. (1977). Lymphoproliferative responses to antigens mediated by human pulmonary alveolar macrophages. *J. Lab. Clin. Med.* **89,** 1326–1332.

Lee, K., Tronchimowicz, H. J., and Reinhardt, C. F. (1985). Pulmonary response of rats exposed to titanium dioxide (TiO_2) by inhalation for two years. *Toxicol. Appl. Pharmacol.* **79,** 179–182.

Lehnert, B. E., and Morrow, P. E. (1985). Association of ^{59}iron oxide with alveolar macrophages during alveolar clearance. *Exp. Lung Res.* **9,** 1–16.

Lehnert, B. E., Valdez, Y., and Tietjen, G. (1989). Alveolar macrophage particle relationships during lung clearance. *Am. J. Respir. Cell. Mol. Biol.* **1,** 145–154.

Lehnert, B. E., Ortiz, J. B., London, J. E., Valdez, Y. E., Clinc, A. F., Sekring, R. J., and Tietjen, G. L. (1990). Migrating behavior of alveolar macrophages during alveolar clearance of light and heavy burdens of particles. *Exp. Lung Res.* **16,** 451–479.

Lewis, T. R., Morrow, P. E., McClellan, R. O., Raabe, O. G., Kennedy, G. R., Schwartz, B. A., Coche, T. J., Roycroft, J. H., and Chhabra, R. S. (1989). Establishing aerosol exposure concentrations for inhalation toxicity studies. *Toxicol. Appl. Pharmacol.* **99,** 377–389.

Lippmann, M., and Timbrell, V. (1990). Particle loading in the human lung—human experience and implications for exposure limits. *J. Aerosol Med.* **3**(Suppl. 1), 155–168.

Mauderly, J. L., Cheng, Y. S., and Snipes, M. B. (1990). Particle overload in toxicological studies: Friend or foe? *J. Aerosol Med.* **3**(Suppl. 1), 169–188.

McClellan, R. O. (1986). Health effects of diesel exhaust: A case study in risk assessment. *Am. Ind. Hyg. J.* **47,** 1–13.

McClellan, R. O. (1990). Particle overload in the lung: Approaches to improving our knowledge. *J. Aerosol Med.* **3**(Suppl. 1), 197–207.

McNulty, M. J., and Reasor, M. J. (1981). Enhanced phagocytic and bacteriocidal activities of phospholipodotic rat alveolar macrophages. *J. Reticuloendothel. Soc.* **30,** 539–549.

Mermelstein, R., Kilpper, R. W., Morrow, P. E., and Muhle, H. (1991). Lung overload, dosimetry of lung fibrosis and their implications on the respiratory dust standard. In *Proceedings Inhaled Particle VII: British Occupational Hygiene Society International Symposium, Edinburgh, Scotland.*

Miller, B. E., and Hook, G. E. R. (1990). Hypertrophy and hyperplasia of alveolar type II cells in response to silica and other pulmonary toxicants. *Environ. Health Perspect.* **55,** 15–23.

Morrow, P. E., Gibb, F. R., Davies, H., and Fisher, M. (1968). Dust removal from the lung parenchyma: An investigation of clearance simulants. *Toxicol. Appl. Pharmacol.* **12,** 372–396.

Morrow, P. E. (1986). The setting of particulate exposure levels for chronic inhalation studies. *J. Am. Coll. Toxicol.* **5,** 533–544.

Morrow, P. E. (1988). Possible mechanisms to explain dust overloading of the lungs. *Fundam. Appl. Toxicol.* **10,** 369–384.

Morrow, P. E., and Mermelstein, R. (1988). Chronic inhalation toxicity studies. In *Inhalation Toxicology: The Design and Interpretation of Inhalation Studies and Their Use in Risk Assessment,* pp. 103–117. Springer-Verlag, New York/Berlin.

Morrow, P. E. (1989). Toxicokinetics. In *Proceedings of the Toxicology Forum. 1989 Annual Summer Meeting,* pp. 87–90.

Morrow, P. E., Muhle, H., and Mermelstein, R. (1991). Chronic inhalation study findings as a basis for proposing a new occupational exposure limit. *Am. Coll. Toxicol.* **10,** 279–290.

Muhle, H., Creutzenberg, O., Bellmann, B., Heinrich, U., and Mermelstein, R. (1990). Dust overloading of lungs: Investigations of various materials, species differences and irreversibility of effects. *J. Aerosol Med.* 3(Suppl. 1), 111–128.

Muhle, H., Bellman, B., Creutzenberg, O., Dasenbrock, C., Ernst, H., Kilpper, R., MacKenzie, J. C., Morrow, P. E., Mohr, U., Takenaka, S., and Mermelstein, R. (1991). Pulmonary response to toner upon chronic inhalation exposure in rats. *Fundam. Appl. Toxicol.* **17,** 280–299.

Nugent, E. M., and Pesant, I. E. L. (1983). Macrophage function in pulmonary alveolar proteinosis. *Am. Rev. Respir. Dis.* **127,** 780–781.

Oberdörster, G., Baumert, H.-P., Hochrainer, D., and Stöber, W. (1979). Clearance of cadmium aerosols after inhalation exposure. *Am. Ind. Hyg. Assoc. J.* **40,** 443–450.

Oberdörster, G. (1988). Lung clearance of inhaled insoluble and soluble particles. *J. Aerosol Med.* **4,** 289–330.

Oberdörster, G., Ferin, J., Gelein, R., Soderholm, S., and Finkelstein, J. (1992). Role of alveolar macrophages in lung injury: Studies with ultrafine particles. *Environ. Health Perspect.*, in press.

Oberdörster, G., Ferin, J., and Morrow, P. E. (1991). Volumetric loading of alveolar macrophages (AM): A possible basis for diminished AM-mediated particle clearance. *Exp. Lung Res.* **18,** 87–104.

Okabe, T., Yorifuji, H., and Murase, T. *et al.* (1984). Pulmonary macrophage: A major source of lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **125,** 273–278.

Patrick, G., and Sterling, C. (1977). The retention of particles in large airways of the respiratory tract. *Proc. R. Soc. London B* **198,** 455–462.

Pickrell, J. A., Snipes, M. B., Benson, J. M., Harrow, R. L., Jones, R. K., Carpenter, R. L., Thompson, J. J., Hobbs, C. H., and Brown, S. C. (1989). Talc deposition and effects after 20 days of repeated inhalation exposure of rats and mice to talc. *Environ. Res.* **49,** 237–249.

Privalova, L. I., Katsnelson, B. A., Osipenko, A. AB., Yushkov, B. N., and Babushkina, L. G. (1980). Response of a phagocyte cell system to products of macrophage breakdown as a probable mechanism of alveolar phagocytosis adaptation to deposition of particles of different cytotoxicity. *Environ. Health Perspect.* **35,** 205–218.

Roy, M. (1989). Lung clearance modeling on the basis of physiological and biological parameters. *Health Phys.* **57**(Suppl. 1), 255–262.

Sibille, Y., Merrill, W., Naegel, G. P., Care, S., Cooper, J. A., Jr., and Reynolds, H. Y. (1989). Human alveolar macrophages release a factor that inhibits phagocyte function. *Am. J. Respir. Cell Mol. Biol.* **1,** 407–416.

Smith, T. J. (1985). Development and application of a model for estimating alveolar and interstitial dust levels. *Ann. Occup. Hyg.* **29,** 495–516.

Snipes, M. B., and Clem, M. F. (1981). Retention of microspheres in rat lung after intratracheal instillation. *Environ. Res.* **24,** 33–41.

Snipes, M. B., McClellan, R. O., Mauderly, J. L., and Wolff, R. K. (1989). Retention patterns for inhaled particles in the lung: Comparisons between laboratory animals and humans for chronic exposures. *Health Phys.* **57**(Suppl. 1), 69–78.

Stahlhofen, W. (1989). Human lung clearance following bolus inhalation of radioaerosols. In *Extrapolation of Dosimetric Relationships for Inhaled Particles and Gases.* (J. D. Crapo *et al.*, Eds.), pp. 153–165. Academic Press, New York.

Stahlhofen, W., Gebhart, J., Heyder, J., Philipson, K., and Camner, P. (1981). Intercomparison of regional deposition of aerosol particles in the human respiratory tract and their long-term elimination. *Exp. Lung Res.* **2,** 131–139.

Stöber, W., Morrow, P. E., and Hoover, M. D. (1989). Compartment modeling of the long-term retention of insoluble particles deposited in the alveolar region of the lung. *Fundam. Appl. Toxicol.* **13,** 823–842.

Stöber, W., Morrow, P. E., Morawietz, G., Koch, W., and Hoover, M. D. (1990). Developments in modeling alveolar retention of inhaled insoluble particles in rats. *J. Aerosol Med.* **3**(Suppl. 1), 129–154.

Strom, K. A., Chan, T. L., and Johnson, J. T. (1988). Pulmonary retention of inhaled submission particles in rats: Diesel exhaust exposures and lung retention model. *Ann. Occup. Hyg.* **32**(Suppl. 1), 645–657.

Strom, K. A., Johnson, J. T., and Chan, T. L. (1989). Retention and clearance of inhaled submicron carbon black particles. *J. Toxicol. Environ. Health* **26,** 183–202.

Vincent, J. H., Johnston, A. M., Jones, A. D., Bolton, R. E., and Addison, J. (1985). Kinetics of deposition and clearance of inhaled mineral dusts during chronic exposure. *Br. J. Med.* **42,** 707–715.

Vostal, J. J. (1986). Factors limiting the evidence for chemical carcinogenicity of diesel emissions in long-term inhalation experiments. Satellite symposium on toxicological effects of emissions from diesel engines. In *Proceedings of the Seventh International Congress of Toxicology. July 26–28. Tsukata Science City, Japan.*

Ward, P. A., Till, G. O., Kunkel, R., and Beauchamp, C. (1983). Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J. Clin. Invest.* **72,** 789–801.

Warheit, D. R., Crandall, E. D., Gillett, N., Phipps, R. P., and Pinkerton, K. E. (1990). Summary of discussions from Session I—Particle–cell interaction–cytology. *J. Aerosol Med.* **3**(Suppl. 1), 57–60.

White, H. J., and Bhagwan, D. G. (1981). Early pulmonary response of the rat lung to inhalation of high concentration of diesel particles. *J. Appl. Toxicol.* **1,** 104–117.

Witschi, H. (1990). Lung overload: A challenge for toxicology. *J. Aerosol Med.* **3**(Suppl. 1), 189–196.

Wright, J. R., and Youmans, D. C. (1991). Surfactant protein SP-A stimulates migration of alveolar macrophages. *Am. Rev. Respir. Dis.* **143,** A314.

Yu, C. P., Chen, Y. K., and Morrow, P. E. (1989). An analysis of alveolar macrophage mobility kinetics at dust overloading of the lungs. *Fundam. Appl. Toxicol* **13,** 452–459.