

ISSUES

Possible Mechanisms to Explain Dust Overloading of the Lungs

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Possible Mechanisms to Explain Dust Overloading of the Lungs. MORROW, P. E. (1988). *Fundam. Appl. Toxicol.* 10, 369-384. This paper briefly reviews the available evidence on dust overloading of the lungs, a condition which has come to the forefront in many recently reported chronic inhalation studies. A general hypothesis is developed that dust overloading, which is typified by a progressive reduction of particle clearance from the deep lung, reflects a breakdown in alveolar macrophage (AM)-mediated dust removal due to the loss of AM mobility. The inability of the dust-laden AMs to translocate to the mucociliary escalator is correlated to an average composite particle volume per alveolar macrophage in the lung. When this particulate volume exceeds approximately $60 \mu\text{m}^3/\text{AM}$, on the basis of a uniform distribution of particles over the AM pool size ($\sim 2.5 \times 10^7$ cells) in the Fischer 344 rat, the overload effect appears to be initiated. When the distributed particulate volume exceeds $\sim 600 \mu\text{m}^3$ per cell, the evidence suggests that AM-mediated particle clearance virtually ceases and agglomerated particle-laden macrophages remain in the alveolar region. This paper considers possible mechanisms why these particle-laden cells are immobilized, viz., one is based on excessive particle-cell, cell-cell chemotactic interactions, and migratory inhibition factors; the other considers the volumetric increase by phagocytized particles, per se, as leading to an inability of the AM to spread and migrate probably through a competitive requirement for surface membrane and cytoskeleton in both endocytotic and migratory functions. © 1988 Society of Toxicology.

BACKGROUND OF DUST OVERLOAD CONCEPT

A substantial number of experimental observations (Adamson and Bowden, 1980, 1981; Bolton *et al.*, 1983; Bowden and Adamson, 1984; Bowden, 1987; Chan *et al.*, 1984; Davis *et al.*, 1978; Ferin, 1972, 1977; Ferin and Feldstein, 1978; Green *et al.*, 1983; Klosterkotter and Buneman, 1961; Klosterkotter and Gono, 1971; Le Bouffant, 1971; Lee *et al.*, 1983; Matsuno *et al.*, 1986; Middleton *et al.*, 1979; Muhle *et al.*, 1987a,b; Shami *et al.*, 1984; Wehner *et al.*, 1983; White and Bhagwan, 1981; Wolff *et al.*, 1985, 1987) has led to the general concept of dust overloading of

the lungs. The essence of this experimental finding is bipartite: (a) long-term exposure to relatively high dust concentrations leads to excessive pulmonary dust burdens whereby the pulmonary clearance of persistently retained particles by alveolar macrophages becomes progressively reduced until it essentially ceases; at this time, lung dust burdens increase linearly at a build-up rate approximating the rate of dust deposition; (b) as excessive lung burdens develop, a number of alterations appear in both the disposition of retained particles and their pattern of induced responses and toxic actions within the lungs. In both regards, interest is mainly with inhaled particles whose retention half-times in

the lungs are measured in weeks, months, or years, and whose toxicity depends upon chronicity.

Thus, breakdown of macrophage-mediated dust clearance creates an artifactual lung condition typified by a sequence of dysfunctional and pathologic changes. While the temporal development of these nonspecific changes may differ with different dusts and the degree of dust overloading, most materials appear to initiate a quantitatively similar pattern of changes, e.g., widespread accumulations of dust-laden macrophages within alveoli; enhanced appearance of lymphoid-associated and interstitial dust deposits; and persistent inflammatory changes with increased epithelial permeability, associated with neutrophil infiltration and activation. These conditions usually progress during chronic exposures until there is a development of alveolitis and granulomatous lung disease, such as fibrosis (Adamson and Bowden, 1981; Bowden, 1987; Hunninghake *et al.*, 1984; Gross, 1967; Pepelko *et al.*, 1980; Reiser and Last, 1986).

The toxicological implications of lung overloading should be obvious. Even many dusts which have generally been recognized as innocuous and considered to fall within the Nuisance Dust classification (ACGIH, 1981), have, under conditions of dust overloading, been shown to produce a diminution of dust clearance and a variety of unexpected toxicological endpoints including tumorigenesis (Brand, 1986; Holland *et al.*, 1986; Lee *et al.*, 1985). Dusts with established toxicities, such as silica, in overload circumstances, have been reported as producing a relatively high incidence of lung cancer: a pathological endpoint seldom seen in man or experimental animals under lesser exposure conditions and lower lung burdens of silica (Groth *et al.*, 1986). Several contemporary papers have pointed out the confounding nature of dust overloading in interpreting the outcome of recent chronic inhalation toxicity studies for diesel particles (McClellan, 1986; Vostal, 1986).

It should be emphasized that lung overloading per se does not depend upon the inherent chronic toxicity of the investigated material. Whether the material is potentially tumorigenic, fibrogenic, or completely benign, dust overloading superimposes its actions of modifying both the dosimetry and the toxicological effects of the test material.

In chronic studies, the incorporation of a surrogate particle, e.g., $^{59}\text{Fe}_2\text{O}_3$, which is known to be cleared from the lungs by alveolar macrophages (AMs) (Lehnert and Morrow, 1985b) has been shown to track the change in retention of the test dust. For this application the animal is exposed to microgram quantities of the surrogate aerosol and its retention is measured independently but concurrently with the test dust. As the lung burden of the test dust becomes excessive and its retention time is prolonged, a concurrent prolongation of the surrogate particle retention has been observed. This circumstance adds credence to the involvement of the AM in the overload process (Morrow, 1986; Muhle *et al.*, 1987a,b; Wolff *et al.*, 1985, 1987).

Several important aspects of dust overloading are still poorly understood. One obvious need is for a better quantitative definition of the overload condition. While the level of dust burden causing overloading appears to be greater than 1–2 mg of a relatively persistently retained dust in the lungs of a Fischer 344 rat, for example (Chan *et al.*, 1984; Wolff *et al.*, 1985, 1987; Muhle *et al.*, 1987a,b), such a dust burden does not produce an all-or-none type of overload response. Rather, the responses and effects produced become more severe as the lung burden exceeds this general level. Chronicity also appears to be a significant factor in its development.

Another important informational deficiency pertains to the general appropriateness of the overload phenomena to all persistent dusts and to other species besides the rat. Since the impact of overloading appears to increase with the absolute dust burden in the lungs, it is important to understand to what

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extent this condition of impaired macrophage clearance is reversible. Intuitively, one would expect recovery to be faster at lung levels which did not cause a cessation of dust clearance.

In any case, dust overloading threatens to compromise many expensive, long-term studies unless there is some reasonable strategy to limit inhalation exposures in ways analogous to those now applied to parenteral and oral administrations involved in other chronic toxicity investigations (National Toxicology Program, 1984; Munro, 1977). Suggestions toward this goal were described in two recent publications (Morrow, 1986; Morrow and Mermelstein, 1987).

Despite informational deficiencies and the lack of any systematic study of dust overloading, there appears to be a plausible mechanistic basis for the overload phenomenon. This paper develops mechanistic hypotheses largely from studies which have attempted to quantify the size and capacity of the pulmonary macrophage pool and from studies which have described the kinetics and responses of AMs during quantitatively described particle exposures (Lehnert and Morrow, 1985b).

STATEMENT OF HYPOTHESIS

The principal overload hypothesis to be developed in this paper proposes that the condition of dust overloading in the lungs is caused and perpetuated by a loss in the mobility of the alveolar macrophage. Moreover, this loss of mobility that impedes macrophage translocation from the lungs is produced by the phagocytosis of excessive amounts of particles, a condition which can be expressed as the cumulative particle volume per alveolar macrophage.

The overall mechanistic hypothesis requires a step-wise development. First, particle build-up and clearance kinetics and how these relate to chronic inhalation exposure conditions will be examined. Second, we will review AM-mediated clearance and apply

known lung burdens associated with dust overload in order to develop the primary hypothesis, viz., that a progressive increase in the cumulative size of particle volume phagocytized leads to AM immobilization. Estimates of the boundary conditions for particle overloading are described. Third, possible bases for the unusual prolongation and ultimate cessation of AM-mediated particle clearance are examined in the context of the loss of translocation mobility of the "overloaded AM."

KINETIC DESCRIPTION OF OVERLOAD CONCEPT

Kinetically, the clearance of dust from the alveolar or pulmonary region of the lungs has usually been treated as a first-order process (Morrow, 1977). This widely applied model is not based on specific clearance mechanisms although it is evident that several known clearance processes are, or closely resemble, single first-order processes. Rather, the model has been used mainly because it provides a kinetically suitable description of pulmonary clearance and a relatively simple dosimetric approach (Morrow, 1973).

In depicting a single-compartment, first-order clearance model (Fig. 1A) d is the rate of pulmonary dust deposition and k is the fractional clearance rate of the lung compartment removal and L is the dust content of the lung compartment. One can see that the build-up rate of L ($\dot{L}$) depends upon the relationship $\dot{L} = d - kL$ mg day⁻¹, where d is expressed as milligrams per day, L is expressed in milligrams and k equals $\ln 2/t_{1/2}$ where $t_{1/2}$ is the retention half time in days; therefore, k is expressed as fraction per day (day⁻¹). When d approximates the value of the product kL , and $\dot{L}$ approaches zero (Fig. 1B), the increasing similarity of absolute deposition and absolute clearance rates leads asymptotically to the steady-state lung burden, L_{ss} . The dust build-up rate before and at the steady-state condition is accordingly expected to resemble that depicted in Fig. 2.

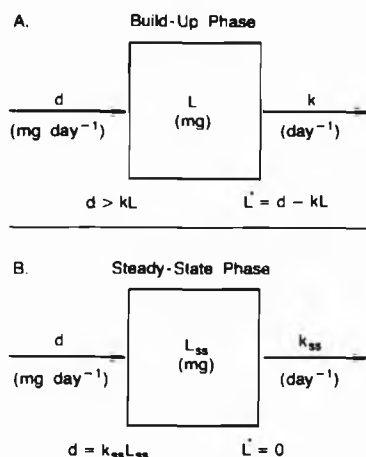


FIG. 1. Build-up and steady-state kinetics of single-compartment lung model employing first-order dust clearance during a chronic dust exposure. (A) The kinetic relationships between dust deposition (d) and clearance (k) are depicted during the build-up phase of dust in the lungs. The build-up rate is described by L . (B) When the dust burden in the lungs achieves a certain level, the amount of dust removed by first-order clearance (k_{ss}) becomes equal to the rate dust deposition (d). This heralds the achievement of a steady-state lung burden (L_{ss}).

This type of build-up curve was nicely demonstrated in the rat, dog, and monkey during chronic studies by Leach *et al.* (1970, 1973) and kinetically inferred by the analyses of coal miner lungs reported by Stöber *et al.* (1967).

It is conventional to accept five retention half-times ($t_{1/2}$) as the approximate time required to produce L_{ss} . In reality five $t_{1/2}$ constitutes only 97% of L_{ss} which, in theory, equals $d(t_{1/2}/\ln 2)$. Keeping the amounts of dust expressed in milligrams and time expressed in days, the predicted value of L_{ss} in milligrams is approximately equal to $1.44 d t_{1/2}$ or can be determined from the ratio: d/k .

The deposition rate d (mg day^{-1}) is obviously determined by the exposure concentration (mg m^{-3}), the respirable dust deposition fraction, and the ventilation of the exposed subject ($\text{m}^3 \text{ exposure day}^{-1}$). For illustrative purposes, we can assume a Fischer 344 rat, during a 8-hr exposure day, ventilates (180

$\text{ml min}^{-1} \times 480 \text{ min}/10^6 \text{ ml m}^{-3}$) or $0.0864 \text{ m}^3 \text{ day}^{-1}$.

Applying a 0.10 rat respirable deposition fraction, the rate of dust deposition (d) will equal the product of the amount of air breathed by the rat times the exposure concentration (C) times this deposition fraction, i.e., $0.0864 \text{ m}^3 \text{ day}^{-1} \times (C) \text{ mg m}^{-3} \times 0.10$. This product, $8.64 \times 10^{-3} (C) \text{ mg day}^{-1}$, equals the value of d . Because chronic dust exposures are usually limited to 5 days a week, we can "adjust" the value of d by $\frac{5}{7}$, so that we can treat the rate of dust deposition as though it pertained to continuous daily exposures. Thus, the adjusted d is equal to $6.17 \times 10^{-3} (C) \text{ mg day}^{-1}$.

The one remaining factor which determines the rate of achieving L_{ss} and its absolute value is the pulmonary retention half-time for the dust. In order to examine the impact of different exposure concentrations on L_{ss} , we will assume a 60-day retention half-time, which means that the clearance rate coefficient, $k = \ln 2/60 \text{ days} = 0.0116 \text{ day}^{-1}$, pertains to the dust.

In the first example, we will consider (C) to be equal to 2 mg m^{-3} . This signifies that the rate of dust deposition, d , equals $6.17 \times 10^{-3} \text{ m}^3 \text{ day}^{-1} \times 2 \text{ mg m}^{-3}$ or $0.01234 \text{ mg day}^{-1}$.

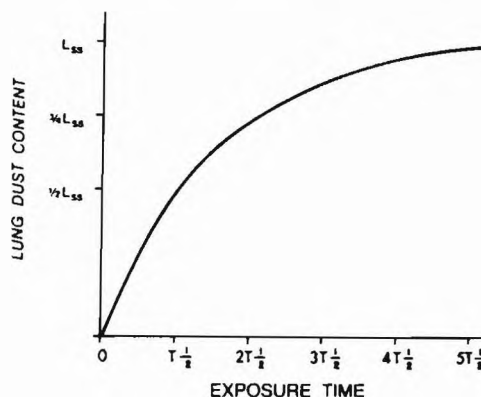


FIG. 2. Build-up rate of lung dust content during clearance inhalation exposure. In this depiction the level of lung dust build-up is expressed as a fraction of the steady-state lung burden (L_{ss}). Time is expressed in terms of the retention half-time of the test dust determined from the clearance rate coefficient (k), that is, $t_{1/2} = \ln 2/k$.

Case 1
Case 2
Case 3
Case 4

* In all cases, the exposure was continuous weekly.

From the data that were obtained when the product of the exposure concentration and the retention half-time was made, it was predicted that the achievement of a steady-state lung burden would require a time for which the lung dust content would be equal to the steady-state lung burden. All other values of L_{ss} would be given in Table 1. The exposure and the incidence of going to the next step in the deposition rate would be 4 assumed build-up lung burden values would be shown.

TABLE 1

INTERRELATIONSHIPS OF RESPIRABLE EXPOSURE CONCENTRATIONS AND DUST RETENTION
IN ACHIEVING STEADY-STATE LUNG BURDENS^a

	Exposure concentration (C) (mg m ⁻³)	Deposition fraction	Deposition rate (d) (mg day ⁻¹)	Retention half-time (days)	Clearance rate (k) (day ⁻¹)	Steady-state lung burden (L _{ss}) (mg)	Time to achieve L _{ss} (days)
Case 1	2	0.10	0.01234	60	0.0116	1.06	300
Case 2	2	0.10	0.01234	120	0.0058	2.12	600
Case 3	20	0.01	0.01234	60	0.0116	1.06	300
Case 4	2	0.10	0.1243	60	0.0116	10.6	300

^a In all examples, the rat is assumed to breathe 0.0864 m³ per exposure day and all exposures are adjusted so as to resemble continuous weekly exposures, as described in the text.

From the lung model (Fig. 1B), we can see that under steady-state conditions, that is, when d is equal to 0.01234 mg day⁻¹, the product of kL_{ss} will also equal this value, therefore, $L_{ss} = 0.01234 \text{ mg day}^{-1} / 0.0116 \text{ day}^{-1} = 1.06 \text{ mg}$. With the assumptions made, the first-order clearance model predicts that in 300 days ($5 \frac{1}{2}$) we will effectively achieve L_{ss} , actually 97% of L_{ss} or 1.03 mg.

If we had chosen a longer retention half-time for the dust, say 120 days, the value of k would be one-half as large, i.e., 0.0058 day⁻¹. All other assumptions unchanged, the value of L_{ss} would be twice as large, 2.12 mg, and it would be effectively reached in 600 days. This and several other changes in assumptions are given in Table 1. Of the examples presented in Table 1, let us focus on case 4 where the exposure concentration is taken as 20 mg m⁻³ and the predicted L_{ss} is 10.6 mg. Available evidence on dust overloading suggests the foregoing method of predicting L_{ss} would not apply since more than 2 mg of dust would be deposited in the rat's lungs within the first 30 days of exposure. In Fig. 3, we see the build-up rate and L_{ss} value associated with the case 4 assumption (Table 1). Additionally, a linear build-up rate, i.e., a constant daily increase in lung dust content associated with an insignificant daily reduction in dust clearance, is shown having a value of 0.1234 mg day⁻¹, the value of d . This latter depiction, in turn, indicates that at 40.5 days, for example, there would be 5 mg of dust in the lungs, slightly

more than predicted by the first-order kinetic model. Instead of reaching 97% of the predicted L_{ss} (10.3 mg) in 300 days, the linear build-up model indicates that this will happen in 83.5 days ($10.3 \text{ mg} / 0.1234 \text{ mg day}^{-1}$) while at 300 days, 37 mg is the predicted value for the lung dust burden, L .

The foregoing development and these simplified estimations are predicated on the assumption that clearance mechanisms other than macrophage-mediated particle removal are relatively unimportant over the chronic exposure time. For instance, estimations of

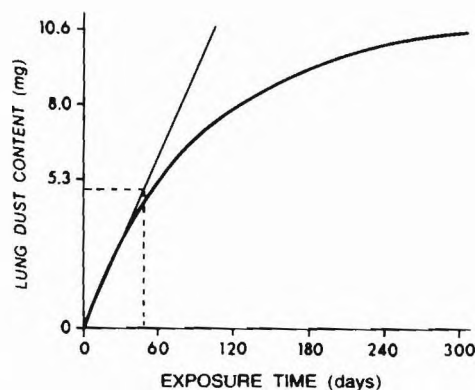


FIG. 3. Case 4 build-up kinetics. In this graphic depiction, both a first-order build-up rate and a linear build-up rate are given. Inasmuch as dust overloading would occur with either build-up rate assumption in less than 30 days of exposure, the linear build-up will be closer to reality throughout the subsequent exposure time, provided all assumptions remain constant.

clearance by dissolution have often yielded relatively long half-times (Mercer, 1967). Such a removal process could account for a ~10% reduction in the lung burden achieved. This would lead to a more-or-less linear build-up rate intermediate to the two curves depicted and a somewhat smaller value for L .

To have avoided a condition of excessive lung levels occurring in the foregoing example (case 4), either the exposure concentration or the respirable deposition fraction would have had to be reduced by about a factor 10 (e.g., case 3 in Table 1), thereby, a predicted L_{ss} of 1.1 mg would be expected and the build-up of dust in the lungs would follow the simple first-order kinetics initially depicted (Fig. 2).

Kinetic descriptions of dust clearance using a single compartment and single or multiple first-order clearance functions have proved both versatile and useful, but they uniformly depend upon the proposition that the value of k does not vary with the size of L . This has led to an alternate approach, for example, represented by Vincent *et al.* (1985) and by Strom and Chan (1984). Both models envision a nonlinear sequestration compartment which has a very small clearance coefficient (long retention half-time). For example, in Strom and Chan's model, the sequestration compartment becomes more dominant as the mass of particles in the macrophage compartment increases. This leads to a redistribution of particles to the sequestration and lymphatic compartments. Kinetically, these nonlinear models with sequestration compartments are capable of describing overload effects by the use of a complex set of differential equations. The power of these models is somewhat offset by their inherent complexity and the recognition of profoundly abnormal events in the lungs induced by excessive dust burdens, including a blockage of macrophage migration and a virtual cessation of AM-mediated dust clearance. Evolution of an abnormal or

pathologic compartment does not befit the usual compartmental concept.

Another version of this approach, is that of Yu and Morrow (1987) which makes use of a simpler nonlinear model, i.e., where the value of k is a function of the magnitude of L . This model has the capability of describing kinetically the prolongation of pulmonary dust retention both as a normal consequence of chronic exposures and as an overload phenomenon without invoking a hypothetical compartment. In this case, "overloading" is an extension of the normal prolongation effect of an increasing lung burden. Linked to this decrease in pulmonary dust clearance with increasing lung burden is a concomitant increase in translocation of dust to the lymphatic drainage and lymphoid tissue (Adamson and Bowden, 1981, 1982; Ferin, 1972; Ferin and Feldstein, 1978; Strom and Garg, 1985; Yu and Morrow, 1987) which also occurs in the condition of dust overloading.

The solubility clearance model proposed by Mercer (1967) is another example of a nonlinear model where the decreased rate of dissolution (clearance) and the increased rate of lymph nodal uptake were both linked to increasing pulmonary dust levels. Mercer's model is focused on clearance by a non-equilibrium dissolution process, whereas the implications of the dust overload concept focus on the capacity of the alveolar macrophage system and its progressive incapacitation, a functional concept readily applied to the nonlinear models of Strom and Chan (1984), Vincent *et al.* (1985), and Yu and Morrow (1987).

THE AM SYSTEM AND DUST OVERLOADING

Studies have shown that the size of the AM population of the rat lungs is altered by the alveolar deposition of dust particles (Brain, 1971; Bowden, 1987; Lehnert *et al.*, 1985c). In a study designed to examine the effects of microgram (27–44 μ g) lung burdens of an in-

nocuous dust on AM response in SPF Long-Evans rats, Lehnert and Morrow (1985a,b), using ^{59}Fe oxide aerosol ($1.6 \pm 0.1 \mu\text{m}$ MMAD) and multiple bronchoalveolar lavages, estimated the AM pool size as 2.14×10^7 cells with a daily output from the rat lungs of $\sim 2.8 \times 10^5$ AM. This latter estimate agrees reasonably well with that made by Masse *et al.* (1977) of 7.5×10^5 AM per day and the AM pool size estimation of Crapo *et al.* (1980) of $\sim 3 \times 10^7$ cells, both investigators using different strains of rats.

The AM study of Lehnert and Morrow (1985b) provided evidence by lavagete analyses that the pulmonary clearance of Fe_2O_3 particles is $>90\%$ pulmonary macrophage-mediated. On the basis that pulmonary retention half-times reported for Fe_2O_3 by several groups (Gibb and Morrow, 1962; Lehnert and Morrow, 1985b; Morrow *et al.*, 1964; Muhle *et al.*, 1987a) range between 53 and 65 days and average around 60 days, and the assumption that Fe_2O_3 clearance is completely due to AM removal, then a clearance coefficient, k , of $\ln 2/60$ days $= 0.0116 \text{ day}^{-1}$ should apply to the fraction of the macrophage pool translocated daily. Averaging the estimates cited regarding AM pool size to 2.5×10^7 cells, then the product, 2.5×10^7 cells (0.0116 day^{-1}), indicates 2.9×10^5 cells are translocated day^{-1} , a value similar to those cited earlier and determined on independent bases.

If we accept a provisional value of 1 mg dust/g of lung tissue as the lowest level producing the overload condition then in the adult Fischer 344 rat of ~ 1.5 g lungs, a 1.5 mg burden is, in principle, relegated to an AM pool consisting of $\sim 2.5 \times 10^7$ cells. This is equivalent to $\sim 6 \times 10^{-8}$ mg dust/AM. Since it is logical to assume that the density of the particle is irrelevant to phagocytosis (Morrow and Mermelstein, 1987), we can re-express the mass of the lung dust in terms of an equivalent volume with a density (ρ) equal to 1.0, thereby, 1 mg of dust is volumetrically equivalent to $1 \times 10^{-3} \text{ cm}^3$. To work with volumetric units more suitable for particles and

cells, we can convert $1 \times 10^{-3} \text{ cm}^3$ to μm^3 by the factor $1 \times 10^{12} \mu\text{m}^3/\text{cm}^3$; thus, 1.5 mg of unit density dust has a composite volume of $1.5 \times 10^9 \mu\text{m}^3$. Allocating this composite particle volume to $\sim 2.5 \times 10^7$ AM pool indicates an average particle volume of $60 \mu\text{m}^3/\text{AM}$ under the initial conditions of excessive lung burdens whereby evidence of dust overloading begins to appear.

Expressing lung burdens volumetrically indicates that TiO_2 , for example, with a $\rho = 4.3$, requires four times the particulate mass burden to bring about the same volumetric loading of the macrophage pool, i.e., 4–8 mg TiO_2/g lung instead of 1–2 mg dust/g lung. This fits the experimental findings with TiO_2 of Lee *et al.* (1985). In retrospect, our initial description of overloading being associated with 1–2 mg dust/g of lung arose from studies of coal, soot, diesel, and copolymer particles, all essentially unit density materials. To avoid the dust density issue, overloading can be expressed as starting at a lung burden of about $1\text{--}2 \times 10^9 \mu\text{m}^3$ particulate volume/g lung ($60 \mu\text{m}^3/\text{AM} \times 2.5 \times 10^7 \text{ AM/lung}$) in the rat lung.

The median volumetric size of a rat AM is approximately $1000 \mu\text{m}^3$ (Dethloff *et al.*, 1987; Lehnert and Morrow, 1984; Strom, 1984); therefore, this $60 \mu\text{m}^3$ particulate volume can be thought of as producing a 6% increase in the average AM volume. Since a $1\text{-}\mu\text{m}$ spherical particle ($\rho = 1.0$) is $5 \times 10^{-13} \text{ cm}^3$ or $0.5 \mu\text{m}^3$, $60 \mu\text{m}^3$ is represented by 120 such particles. By analogy, for $3\text{-}\mu\text{m}$ particles, $60 \mu\text{m}^3$ is represented by 4 or 5 such particles. This and other volumetric computations in this paper neglect the complexity of void spaces associated with close packing of spheres: these could, under some conditions, increase the composite particle volume of uniform spheres by about one-third or more (from $1 - 4\pi/18$ to $1 - 4\pi/24$) (Stöber, 1972). Another neglected complexity pertains to the range of AM cellular volumes. As Strom (1984) reported, AM volumes, even under control circumstances, are highly skewed and

include some cells with volumes greater than $4000 \mu\text{m}^3$ volume.

In a recent review by Bowden (1987), several collaborative studies with Adamson (Adamson and Bowden, 1980, 1981, 1982; Bowden and Adamson, 1984) are described which analyzed the AM response in mice given various particle sizes and burdens. Recruitment of AMs was found to be related to the size and number of the administered particles. At constant lung loading, $0.03\text{-}\mu\text{m}$ carbon particles induced a three-fold greater increase in the lavageable AM population than uniform latex particles of $1.0\text{-}\mu\text{m}$ diameter, with $0.1\text{-}\mu\text{m}$ latex particles being intermediate in their AM recruitment.

Adamson and Bowden (1981) reported that in the mouse lung, approximately 10^{10} particles of $0.03 \mu\text{m}$ were needed to stimulate division of interstitial cells and produce a concomitant increase (up to a factor 10) in lavageable cells (AM) over a control rate of $\sim 2.6 \times 10^5$ AMs per day in unexposed mice. If substantially larger numbers of particles were instilled in the mouse lung, for example, 8×10^{13} carbon particles of $0.03\text{-}\mu\text{m}$ size or 8×10^9 latex particles of $1.0\text{-}\mu\text{m}$ diameter, no further elevation in lavageable AMs occurred signifying a "saturation" of AM recruitment. The differences seen in macrophage recruitment between the largest ($1.0 \mu\text{m}$) and smallest ($0.03 \mu\text{m}$) particle investigated was about a factor 3, with the maximal effect produced within the first postinstillation week.

For purposes of this discussion, data for the $1\text{-}\mu\text{m}$ particle will be used since this particle size is typical of dusts used in inhalation studies and is close to the particle size used by Lehnert and Morrow (1985a,b) for AM kinetics. If we accept 8×10^5 AM as representing an average, sustained daily translocation rate from the dust-exposed mouse lung (Adamson and Bowden, 1981) and assume that the lavageability of mouse AMs is comparable to that determined for the rat, then the activated AM pool size for the mouse would be about 8×10^7 cells.

The composite volume for 8×10^9 uniform particles of $1.0\text{-}\mu\text{m}$ -diameter is $4 \times 10^9 \mu\text{m}^3$. From this calculation and the estimated AM pool size, ($4 \times 10^9 \mu\text{m}^3 / 8 \times 10^7$ AM), we obtain an estimate of $50 \mu\text{m}^3/\text{AM}$ when the AM recruitment system is characterized by Bowden as "saturated" (Bowden, 1987). The similarity of this composite particle volume per macrophage to the $60 \mu\text{m}^3/\text{AM}$ value obtained on the basis of rat studies is apparent.

Bowden stressed that this "saturation" level of innocuous particles was associated with a general pulmonary inflammatory response, an increased likelihood of free particles in the *interstitium*, and increased localization of particles in peribronchial and perivascular connective tissue (Adamson and Bowden, 1980, 1981, 1982; Bowden and Adamson, 1984). These responses are similar to those seen with particle overloading and appear to support the general hypothesis that the progressive prolongation of particulate retention derives from a combination of events which includes the apparent inability of the AM system to adapt to excessive dust burdens.

The mean $50\text{--}60 \mu\text{m}^3/\text{AM}$ volume change obtained by the foregoing calculations infers each AM phagocytizes the same volume (number) of particles. This is clearly not the case as Kavet *et al.* (1978) and others have shown (Lehnert and Morrow, 1984; Lehnert *et al.* (1985d). Frequency distributions of particles/AM vs percentages of the AM populations have been found to be skewed and sometimes Poisson-like (Kavet *et al.*, 1978). At early times after AMs are exposed to uniform latex particles in either *in vitro* or *in vivo* systems (the latter being assessed by lavaged AMs), there is clearly a broad distribution of particle numbers per AM. For example, Lehnert *et al.* (1985d) intratracheally administered 4×10^8 uniform latex particles of $1.9\text{-}\mu\text{m}$ -diameter to each of five Sprague-Dawley rats. After 24 hr, lavaged AMs (pooled data) demonstrated that $\sim 99\%$ of the particles were cell-associated. Moreover, at this time a mean of $\sim 35\%$ of the lavaged AMs contained

no particles; 18% contained 1-5 particles/AM; 12% contained 6-10 particles/AM; 6% contained 11-15 particles/AM; and 3% contained 16-20 particles/AM. A substantial fraction, ~26%, of the lavaged AMs contained more than 20 particles. Subsequently, Lehnert reexamined this portion of the frequency distribution (1987). The extended grouped data were as follows: approximately 5% of the AMs contained 21-30 particles/cell; ~5% contained 31-40 particles/AM; ~5% contained 41-50 particles/AM; ~3% contained 51-60 particles/AM; ~3% contained 61-70 particles/AM; ~2% contained 71-80 particles/AM; and ~1% contained 81-90 particles/AM. The remaining categories 91-100, 101-110, 111-120, 121-130, and >130 particles/AM were associated with mean percentages of 0.8, 0.2, 0.2, 0.2, and 0.1, respectively.

A rough estimate based upon these original and extended data indicates that the average number of 1.9- μm particles, each with a 3.6 μm^3 volume, per AM was of the order of 15. Furthermore, this distribution signifies that ~73% of the AM had up to 60 μm^3 of 1.9- μm -diameter particles; 37% of the AM were distributed within the overload range, i.e., contained between 60 and 500 μm^3 /AM. Clearly, these highly skewed distributional data are not Poisson-like.

Instead of visualizing the overload process as affecting all AMs uniformly, it is probably more realistic to envision an increasing fraction of the AM population reaching some composite - particulate - volume limitation which results in a reduced ability to clear particles. With a chronic particle exposure, the increase in lung dust burden results in a progressive increase in the immobilized AM population until ultimately all cells reach this volumetric limitation and AM-mediated clearances ceases.

There is only limited evidence on the magnitude of the lung burden bringing about a virtual cessation of AM-mediated clearance. Such data indicate that a 10-fold greater dust burden is required than is present when over-

loading begins to bring about a significant prolongation of particulate clearance (Chan *et al.*, 1984; Muhle *et al.*, 1987b). Expressed in average terms, the volumetric limitation would constitute at least 600 μm^3 /AM. For 1.9- μm -diameter uniform microspheres, this would be equivalent to ~170 particles per cell, again neglecting the void-space volume which becomes more and more important with increasing numbers of phagocytized particles. For this specific case, adding the void-space volume to the composite volume of 170 uniform 1.9- μm particles/AM might exceed 780 μm^3 /AM, or conversely, only 150 such particles would be needed to exceed the 600 μm^3 /AM volumetric limitation if close packing occurred.

With this restatement of dust overloading, it is interesting to consider the study of Snipes and Clem (1981) with 3-, 9-, and 15- μm diameter polystyrene particles simultaneously administered intratracheally into the lungs of Fischer 344 rats. Polystyrene particles of each size were tagged with a different radionuclide so external counting was useful for determining the respective retention half-times.

Fifty micrograms (~1.7 $\times 10^6$ particles) of 3- μm microspheres was found to be cleared biphasically with the slow (alveolar) half-time slightly longer than that for iron oxide, 69 vs 60 days, respectively. When 5 μg (~2 $\times 10^5$ particles) of 9- μm polystyrene microspheres were administered, the slow clearance phase had a 580-day half-time. Each of these 9- μm -diameter particles has a 382 μm^3 volume. By virtue of the low (0.01) ratio of particles to AM cells (2 $\times 10^5$:2 $\times 10^7$), only a fraction of the AM cells could phagocytize either 1 or 2 of these particles, but either event would be expected to lead to a prolongation of retention and this was found.

For the 15- μm -diameter polystyrene microspheres, except for an initial rapid clearance of ~14% of the instilled particles probably due to entrapment of particles in small airways, the alveolar clearance phase was immeasurable. Each of these 15- μm particles has a 1.77 $\times 10^3$ μm^3 volume which is larger

than the volume of the average AM and greatly exceeds the estimated overload limit for any cell, creating a virtual "all or none" situation as far as AM migration is concerned; hence, no AM-mediated clearance should occur irrespective of the postexposure time, and this was found.

The attainment of the condition of overloading which is termed the "volumetric limit" implies a loss of AM mobility, but not necessarily a concomitant loss or reduction of phagocytic function. Histological data do not reveal what the status of the dust-laden AMs may be in this regard. During the development of the overload condition, the ability of the pulmonary macrophage to phagocytose particles which are not acutely cytotoxic does not appear to be impaired, since alveoli are regularly seen with particle-laden macrophages. Since these accumulations of particle-laden cells persist, it is logical to assume that normal AM cytolysis occurs with prompt reengulfment of released particles by new AMs. This condition is apparently not remedied with time if the dust exposure continues. Whether the condition reverses, how fast and to what extent, after dust-loading ceases has not been determined.

Since the overload condition is sustained during chronic inhalation exposures of many months' duration, one can postulate that the inability of the dust-laden macrophage to translocate from the lungs is the critical feature of the condition. That the mobility of the pulmonary macrophage must be adversely affected by the excessive dust loading is clearly inferred, but whether this is a direct or indirect effect on the AM cannot be deduced from available data.

FACTORS AFFECTING THE MOBILITY OF THE AM TO TRANSLOCATE FROM THE LUNGS

Although much investigative effort has been directed at phagocytic mechanisms, particularly at the role of chemotactic factors

and opsonins, AM activation, AM phagocytic receptors, nonspecific phagocytosis, and various cell-cell interactions (Silverstein, Steinman, and Cohn, 1977; Brain, 1986; Jones, 1984; Musson and Henson, 1984) relatively less fundamental information has been acquired on macrophage mobility *per se*, and very little is known about AM mobility in the context of translocation from the lungs.

At present, two general concepts prevail for AM translocation from the alveoli to the bronchial tree: this appears to be the most widely accepted and dominant translocation pathway for AMs. First, there is the random-walk concept that implies a stochastic basis for a cell to encounter the active translocation region of mucociliary transport, commencing at the level of the terminal bronchioles. The second postulated concept is that of directed migration through the transitional airways to the same "mucociliary escalator." In the latter regard, the AM migration is presumed to be due to either (a) the directed motion of the alveolar fluid lining layer on which the AMs are passively transported or (b) a chemoattractant gradient which directly polarizes and facilitates AM migration along the alveolar epithelial surface.

The first of the concepts of directed migration probably originated with the view of a continuous capillary effusate into the alveoli together with Macklin's description of "sumps" (Macklin, 1955). This concept was furthered by the intuitive model of a respiratory-driven "ebb and flow" for the superficial alveolar fluid lining layer (Gross, 1953; Kilburn, 1974). Unfortunately, the best available evidence refutes the existence of a normal capillary effusate on the alveolar surface and supports the viewpoint that the alveolar surfactant-hypophase layer is generally less than $0.1\ \mu\text{m}$ thick on the alveolar surface and discontinuous (Gil, 1985). Lung surfactant cyclically forms multilayers and then re-spreads, *in situ*, during breathing (dynamic compression and relaxation) and a continuous alveolar lining layer is not required for

appropriate reduction of surface tension (Notter and Morrow, 1975; Notter and Finkelstein, 1984).

Of these several concepts, therefore, directed migration by chemotactic factor(s) seems to be the least contentious. Kinetically directed and random migration by AMs would both resemble first-order processes, i.e., a constant fraction of the AM pool would translocate per unit time. However, the existence of powerful and multifarious chemoattractants seems to provide a basis not only for chemotactic particle-cell interactions leading to phagocytosis, but also for modifications of cell-cell interactions and of AM mobility. Consequently, alterations in cell mobility accompanying particle overloading can be hypothesized as based on excessive elaboration of these factors.

Among the factors which have been demonstrated to induce inflammation and increase epithelial permeability, affect cell adherence, enhance the formation of AM clusters and granuloma, and inhibit AM migratory activity are certain lymphokines, e.g., macrophage migratory inhibition factor (MIF), and several glycoproteins elaborated by AMs and other cells, such as fibronectin and colony stimulating factors (CSFs) (Burgess *et al.*, 1977; Dauber and Daniele, 1980; Fowles *et al.*, 1973; Hunninghake *et al.*, 1984; Kradin *et al.*, 1986; Martin *et al.*, 1984; Metcalf, 1985; Reiser and Last, 1986; Rocklin, 1974; Warr and Martin, 1973). In the latter group, M-CSF, for example, has important actions on AM adherence and self-agglutination and on the proliferation and activation of AMs and neutrophils.

Both AMs and neutrophils produce prostaglandins and activated AMs affect phospholipid production of many molecules with suppressor feedback actions on the AM, leading Metcalf (1985) to suggest an internal self-regulation whereby, *inter alia*, the level of mature AMs controls new AM recruitment. Reiser and Last (1986) used this same information, including some data on lymphokines,

e.g., MIF, to propose a basis for increased cell matrix reactions and granuloma formation.

Collectively, the actions of these chemoattractants and activators and modulators of AM function fit the phenomenologic information on particle-laden, aggregated, immobilized AMs observed during particle overloading. Nevertheless, the evidence that these factors, mainly studied in *in vitro* systems, explain the progressive cessation of AM-mediated particle translocation from the lungs is entirely circumstantial.

It is conceivable that the foregoing factors come into play only in relation to the "saturation" effect on AM recruitment described by Bowden (1987) and in terms of the AM agglutination and granuloma formation. Under these conditions, the AMs may be physically unable to spread and migrate, notwithstanding the possible persistence of a normal or enhanced chemoattractant gradient for directed AM translocation. It is likely that control of the AM translocation process is complex and that all of these speculations regarding AM mobility vary greatly in their relevance.

It seems equally plausible to imagine that the failure of the engorged, particle-laden macrophages to possess normal motility may be due to the volumetric distortion induced in these cells with a concomitant reduction in available cell surface for spreading and to phagolysosomal interactions with the cytoskeletal system (Trotter, 1981; Cain and Kraus, 1981). In other words, a completely mechanical basis for AM immobilization seems possible based on the dual requirement of cell surface membrane and cytoskeleton for both migration and endocytosis (Aggeler and Werb, 1982).

In Fig. 4, we see an example of what might be involved. A fully spread macrophage which may be typical of a migrating cell is seen. On the basis of the field magnification, we can estimate this cell's surface area as $\sim 1250 \mu\text{m}^2$, which is about 2.6 times more surface membrane than a "smooth" AM with a $1000 \mu\text{m}^3$ volume would possess ($1250 \mu\text{m}^2/483 \mu\text{m}^2$). The spherical cell volume of

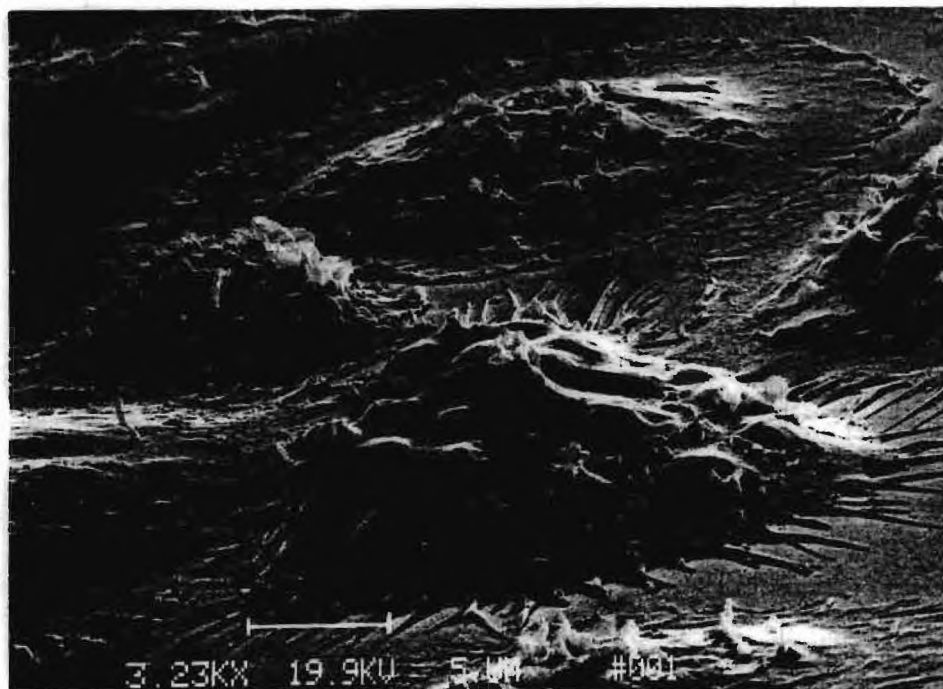


FIG. 4. Alveolar macrophage spreading. These rat alveolar macrophages in this photomicrograph were taken from a cell-particle (sheep erythrocytes) suspension and allowed to adhere to a substrate before fixation and viewing by scanning electron microscopy (SEM). In this selected field, at least one fully spread AM appears almost devoid of particles. The foreground AM, juxtaposed by two other macrophages, appears to have spread less and the surface of its spherical segment is smooth except for many proturbances caused by large numbers of phagocytized erythrocytes. (Photomicrograph provided by Dr. B. Lehnert.)

1000 μm^3 assigned the average AM is based on a displaced volume measurement which is relatively insensitive to the presence of a normally ruffled surface membrane. Hence, we can hypothesize that this "stored" surface membrane is available for phagocytosis and especially for migratory spreading in a somewhat competitive fashion. The AM in the foreground of Fig. 4 is engorged with many sheep red blood cells (>80), almost devoid of ruffles, and appears to have a limited ability to spread. Although SEM images of fixed cells are not dimensionally reliable, this micrograph possibly demonstrates features which may affect the ability of the cell to migrate from the alveolar spaces to the mucociliary region.

Perhaps even more compelling than the surface membrane competition, is that docu-

mented for endocytosis, cell adhesion, spreading, and locomotion of the AM in relation to its cytoskeletal system (Aggeler and Werb, 1982).

Whatever the mechanistic basis for the inhibition of the AM to translocate from the alveolar region, one can deduce that the volumetric "limitation" of 600 μm^3 or thereabouts can result from the phagocytosis of many small particles or of a single large particle such as a 15- μm diameter latex sphere. In other words both the induction of dust overloading and the ultimate breakdown of AM-mediated particle clearance should be considered both as a particle size effect such as that demonstrated by Snipes and Clem (1981) and in terms of a composite volume of phagocytized particles discussed here in relation to excessive particulate burdens.

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SUMMARY AND CONCLUSION

A general mechanistic hypothesis is presented on how and why excessive particle loading brings about a debilitation of AM-mediated particle clearance. The general hypothesis contains several secondary hypotheses; specifically, that particle overloading occurs when a certain cumulative or composite particulate volume is reached in the alveolar macrophage, and that this, in turn, results, directly or indirectly, in the loss of AM mobility and AM-mediated particle transport. The capability of the lung to clear particles, even of low inherent toxicity, is an important defense mechanism. The longer insoluble particles reside in the lung, the more opportunities exist for adverse developments. Accumulation of sufficient number of persistently retained particles may lead to a variety of adverse effects including, pneumoconiosis, hypersensitivity pneumonitis, and tumorigenesis. While it is clear that retardation of particle clearance represents a departure from normal behavior, it is unclear whether lung overloading or in the extreme, cessation of particle clearance by itself represents a toxic endpoint or whether it merely leaves the lung more susceptible to infection and other sources of injury. While this general picture is derived from limited experimental data, it appears to provide coherence to an assortment of highly germane, but seemingly isolated experimental results. In any case, the main features of the hypothesized mechanistic basis for particle overload proposed are subject to experimental refutation or confirmation and, at least, should stimulate the development of additional evidence or alternative explanations for our present, all-too-limited understanding of the phenomena of dust overloading.

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