Smoking Impairs Long-Term Dust Clearance from the Lung

Abstract. *The time for the long-term clearance of dust from human lungs was measured. Three heavy cigarette smokers and nine nonsmokers inhaled a harmless trace amount of magnetic dust, Fe_3O_4. From periodic measurements with a sensitive magnetic detector of the amount of this dust remaining in the lungs, a clearance curve was determined for each subject. This magnetic tracer method allows clearance to be safely followed for a much longer time than with radioactive tracer methods. The dust clearance in the smokers is considerably slower than in the nonsmokers. After about a year, 50 percent of the dust originally deposited remained in the lungs of the smokers whereas only 10 percent remained in the lungs of the nonsmokers. The smokers therefore retained five times more dust than the nonsmokers. This impaired clearance of Fe_3O_4 suggests impaired clearance in smokers of other dusts, such as toxic occupational and urban dusts. The higher retention of these dusts may contribute to the higher incidence of lung diseases in smokers.*

Inhaled dust and smoke can cause or aggravate lung disease. Consequently, increasing attention is being given to the ways in which deposited particles are cleared from human lungs and to the identification of toxic particles. Dust is removed from human lungs by several different mechanisms (*1*). Particles deposited in the airways are carried on a moving mucociliary carpet to the throat, swallowed, and eliminated through the gastrointestinal tract in a day or so (short-term clearance). Particles deposited deeper, in alveoli, are engulfed by macrophages and are cleared more slowly, in times ranging from days to months or more (long-term clearance). Some particles may penetrate connective tissue or enter lymph nodes and persist even longer.

Cigarette smoking has been documented as a cause of cardiopulmonary disease. One mechanism by which it could cause disease is by altering the dust clearance of the lung and thus aggravating the effect of other toxic particles on the lung. We report here the first long-term (1 year) study comparing the dust clearance from the lungs of human smokers and nonsmokers. A magnetic tracer dust is used as a new technique. Our study shows that cigarette smoking slows the dust clearance from the lung, thus increasing the amount retained.

Numerous studies of short-term dust clearance from the respiratory tracts of animals and man have been carried out. In most, radioactively tagged dusts were used. The reports of smoking effects on short-term dust clearance in animals (*2*) and man (*3*) are inconsistent; some report that smoking accelerates short-term clearance, whereas others describe a slowing. The effect of smoking on the initial deposition pattern complicates the interpretation of many of these studies (*3*). In long-term clearance studies in animals, dusts tagged with long-lived radioisotopes have been used; clearance half-times of Fe_2O_3 dust measured in dogs were usually found to be about 70 days (*4*). Smoking depressed the clearance of SiO_2 in rodents (*5*), whereas in dogs it had little effect on the clearance of Fe_2O_3 or Cr_2O_3 (*6*).

There have been fewer studies of long-term clearance from human lungs, because of the hazard of long-lived radioactivity. In the radioactive studies that have been done (*7*), dusts usually tagged with either ^{59}Fe or ^{51}Cr were used (half-lives of 28 and 45 days, respectively). The clearance half-times of these dusts in the lung were found to be about 70 days; the duration of each study was usually 60 days. Smokers were involved in only one study (*8*); ^{198}Au (half-life of 2.7 days) was used to measure the long-term clearance in a group of 20 subjects containing an unspecified number of smokers; they were studied for a period of 30 days or less. Most of the nonsmokers showed a clearance half-time of about 60 days, whereas the smokers showed a longer but unspecified half-time.

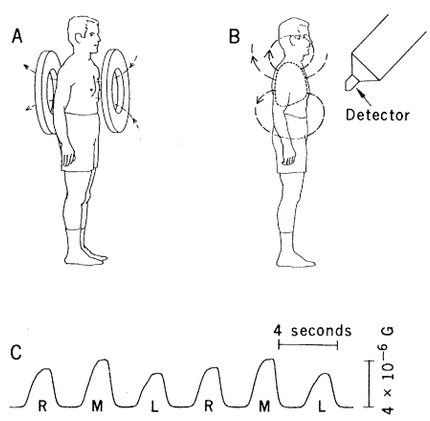

Fig. 1. The method of measuring the amount of Fe_3O_4 in the lungs. (A) The subject is magnetized by an external field generated by two coils. (B) The magnetized particles in the lung produce a remanent field around his torso. He is ready to begin his far-near-far motion at the SQUID detector, located in the tail of the cryogenic container. Not shown here is the spacer, which limits his closest approach to 15 cm. (C) The detector output for the three chest points: right nipple (*R*), middle (*M*), and left nipple (*L*). Two sequences are shown, recorded 2 minutes after magnetization.

In contrast to the radioactive method, our use of a ferromagnetic dust represents a safe, noninvasive approach; the clearance can be followed for a much longer time. The magnetic dust we chose, Fe_3O_4 (magnetite), is harmless in small amounts (*9*) and insoluble at physiological *p*H's. To obtain a clearance curve, periodic measurements are made of the amount of the Fe_3O_4 dust remaining in the lungs after a single session of inhalation. For each measurement, the particles in the lungs are first magnetized with an external magnetic field. After the external field has been removed, the field produced by these magnetized particles, called the remanent field, is measured by a magnetic detector. The reports defining this technique (*10*) showed that as little as 0.02 mg of Fe_3O_4 can be measured with a sensitive magnetic detector called the SQUID (superconducting quantum interference device), when used in a magnetically shielded room. A more convenient (and less expensive) flux-gate detector can be used without shielding and can detect about 0.2 mg of Fe_3O_4 in the lungs. The deposition in the lung of 1 mg of Fe_3O_4 is sufficient to describe a clearance curve with the SQUID. For measurements with the flux-gate detector, 2 mg or more are necessary, depending on the accuracy desired. For the measurements reported here, we used a SQUID in the shielded room at the Massachusetts Institute of Technology (*10*).

One phenomenon seen only with magnetic dust in the lungs is called "relaxation"; after magnetization, the magnetic field over the chest produced by the particles decreases continuously, dropping to about 15 percent in 1 hour. This is due to random, small rotations experienced by the particles in the lungs, which cause a reduction in the vector sum of the magnetic fields of the individual particles. Whenever the amount of dust in the lung was to be measured, we made the field measurements soon after magnetization and for maximum accuracy extrapolated the relaxation curve back to zero time.

Nine nonsmoking and three heavy-smoking male subjects (each subject smoked more than $1^1/_2$ packs of cigarettes per day) between the ages of 21 and 45 were chosen for these clearance measurements. Pulmonary function tests (*11*) were performed by each subject; the results fell within two standard deviations of predicted values, and we concluded that each subject lacked serious pulmonary disease. Each subject was al-

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so screened for previous magnetic contamination. We generated the dust by dispersing powdered Fe_3O_4 (Fisher I-119, 74192), using a fan in a wooden box. A tube connected the box through a fine-mesh filter and a one-way valve to a mouthpiece, which permitted inhalation from the box and expiration into a plastic bag. Dust at the mouthpiece had a mass median aerodynamic diameter of 2.8 μm with a geometric standard deviation (σ_g) of 1.4.

To find out if clearance was sensitive to the particle deposition pattern within the respiratory tract, the breathing of each subject during the exposure was done in one of two ways: in the first, the tidal volume was 2 liters and the frequency was three breaths per minute (deep-slow); in the second, 0.4 liter and 48 breaths per minute (shallow-fast). We monitored the tidal volume with transducers attached to the torso; the frequency of breathing was guided by a metronome. The shallow-fast pattern was expected to produce primarily more airway deposition, and the deep-slow pattern primarily alveolar deposition.

To determine each point on a subject's clearance curve, it was first necessary for him to change into clothes containing no magnetic material. The subject was then magnetized (Fig. 1A) by an external field having a strength of 660 G. (The earth's field is about 0.4 G, and the field at a horseshoe magnet might be 1000 G or more.) The field was uniform over the lung with less than ± 10 percent variability and was applied for 20 seconds in order to give the particles time to rotate in the viscous medium of the lung and align themselves with the external field (*10*). After magnetization, the subject quickly went into the shielded room and began to change his body positions at the detector; these changes produced measurements at one of three points of interest on the chest. For each measurement, the subject first stood out of range of the detector (far) (Fig. 1B). He then moved inward and placed one of the chest points close to the detector (near) and then stepped back again (far). The detector responds only to the horizontal component of magnetic field normal to the subject's chest. It has a bell-shaped response curve in angle, with the maximum at the 0° line (this detector axis is normal to the subject's chest) and a half-maximum at about ± 22°. A spacer was generally used between the subject and the detector, which limited his closest approach to 15 cm; this allowed a broad view of the lung, covering about ± 6 cm from the center to half-maximum.

The measurements began about 20 seconds after magnetization. During the first 2 minutes the remanent field at the first chest point (M), located midway between the two nipples, was repeatedly measured. This yielded an accurate relaxation curve, allowing an extrapolation back to zero time. After 2 minutes, measurements were also made at the right (R) and left (L) nipples. For the data to be valid the value at M must always be higher than at either R or L because the detector views more dust at M. This is seen in Fig. 1C, which also shows the good repeatability of the second sequence. These R-M-L sequences were periodically recorded for about an hour. They were interspersed with a complete mapping of the chest and abdomen without the spacer, to determine the general distribution of magnetic particles in the torso; this was an added check against artifact. For example, a speck of magnetized steel (from canned food), if present in the abdomen, could produce a magnetic field extending up to the chest and an error in the lung measurement; such incidents were rare.

Measurements of the amount of Fe_3O_4 in the lung were made several times during the first week after the inhalation. The purpose was to determine the starting point on the long-term clearance curve, that is, the amount of Fe_3O_4 present after the completion of any short-term clearance. Thereafter, measurements were made every 2 or 3 weeks for about 6 months, then less often until about 12 months. Measurements in the smokers were made for only 11 months because these subjects entered the program some weeks after the nonsmokers.

The smokers showed a much slower dust clearance than the nonsmokers (Fig. 2A). After 11 months, approximately 50 percent of the dust remained in the lungs of the smokers, whereas only about 10 percent remained in the lungs of the nonsmokers. The smokers, therefore, retained about five times more dust than the nonsmokers. Since the curves appear to be leveling off over time, we expect the factor of 5 will be maintained or will perhaps increase.

The spread of long-term clearance

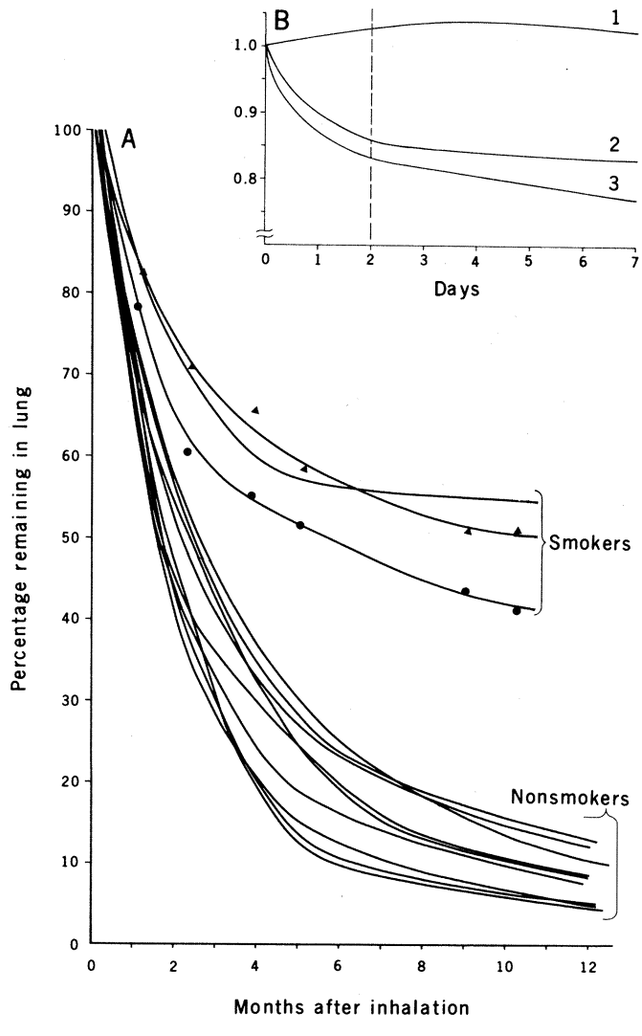

Fig. 2. (A) Long-term clearance curves. After 11 months, the smokers retain in the lungs about five times more Fe_3O_4 dust than the nonsmokers. Experimental points are shown on two curves only, to indicate typical scatter. The 100 percent point of each long-term curve is begun at about the maximum point on the subject's short-term curve, after 2 days (which is after the completion of any short-term clearance). (B) Short-term clearance data for the first 7 days after inhalation. The vertical scale is relative to the amount of dust initially deposited, immediately after inhalation. Curve 1, nonsmokers, deep-slow; curve 2, smokers, shallow-fast; and curve 3, nonsmokers, shallow-fast. The three curves are smoothed-out averages and are representative of all the subjects in that group.

within each of the two groups is seen to be small compared to the difference between the groups. The shapes of individual nonsmoker curves are all similar; the average half-time is about 70 days, but the curves are not exponential. The coherence of these curves indicates that dust clearance in humans can be readily followed for a year or more with the use of a magnetic tracer and can be quantified. In general, the curves obtained for healthy nonsmokers provide a good data base for comparison with abnormal cases. The smokers' curves are not as coherent; they diverge somewhat, and after several years there may be substantial differences in the amounts of dust retained.

For plotting the short-term data only (Fig. 2B), the subjects were divided into three groups. The first group consisted of four nonsmokers who breathed deep-slow (curve 1; the long-term curves of these subjects are those which rank 2, 6, 7, 8, counting down at the right end). The second group consisted of the three smokers who breathed shallow-fast (curve 2). The third group consisted of the remaining five nonsmokers who breathed shallow-fast (curve 3). The fast decline of the lower two curves during the first 2 days shows short-term clearance, as expected; with shallow-fast breathing, when airway deposition is more probable, short-term clearance is more prominent. However, curve 1 shows a rise. This cannot be due to an increase of total magnetic dust in the lung; hence, it must be an artifact produced by the method of measurement. We explain the rise as follows: from a study of the torso maps without the spacer, and of R-to-M and L-to-M differences, it appears that the dust redistributes during the first few days. The detector, because of the bell-shaped response curve, does not view the entire lung uniformly, even with the torso set back by the spacer. When aimed at M, it "sees" the center of the lung field somewhat more than when aimed at the L or R regions; hence, if there were a net inward movement of dust during the first few days (and the three curves in Fig. 2B are heavily weighted with M data), a small rise in the measured amount would be seen, as is the case here. Accurate measurements of the distribution of Fe_3O_4 in the lungs of a different subject, taken by us in an earlier study (*12*), indicate that there is indeed a more central pattern as time passes, which supports our explanation.

Consideration of the short-term clearance data guided us in the selection of a starting point for the long-term clearance curves. The maximum value observed for each subject at more than 2 days after the exposure was used as the 100 percent reference point for this long-term curve. We believe this choice of starting point is best since it largely eliminates the effect of a differing deposition pattern or short-term clearance. In any case, even if the long-term data are plotted relative to the initial deposition or if the curves are moved either way a few days, the dramatic separation between smokers and nonsmokers remains.

There is, of course, the problem of generalizing the results from the data of only three smokers. We have repeatedly examined the technique and the data and can find no artifact that would account for any differences between the smoker and the nonsmoker groups. As examples, we have looked for group differences of age and of method of inhalation; we can find none. The only significant difference between the groups is the fact of smoking. On the other hand, there are auxiliary data from this study, other than clearance data, showing that the lungs of the three smokers handle dust differently from those of the nonsmokers. These are the relaxation curves; relaxation is much more rapid in the lungs of smokers. For example, 20 minutes after magnetization the field from the lungs of the smokers had decreased to 8 percent in contrast to 25 percent for the nonsmokers. Moreover, the relaxation varied with location over the lungs of smokers in contrast to relatively uniform relaxation for the nonsmokers.

Our data indicate, therefore, that smoking impairs the long-term clearance of Fe_3O_4 dust from the lungs. These data, accumulated over a year, confirm the qualitative result of the 1-month study (*8*). Our data also imply that smoking retards the clearance of other dusts, including those that are toxic. This may help explain some epidemiological findings, in particular, those of Selikoff *et al.* (*13*) who have reported that asbestos workers who smoke cigarettes had a 90-fold greater risk of dying of bronchogenic carcinoma than nonsmoking asbestos workers. Wagoner (*14*) has reported a similar synergism between smoking and exposure to radon daughters in uranium miners. We do not suggest that an impairment of alveolar clearance by smoking is the only explanation for this synergism. For example, carcinogenic and irritant chemicals in tobacco may act cooperatively with carcinogens in the work environment and enhance their effect on the lungs. As another example, occupational dusts may serve as a more effective vehicle for the delivery of cigarette smoke carcinogens to the lungs. Nonetheless, we feel that impairment of alveolar clearance caused by smoking is an important effect and worthy of further study.

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9. The safety of trace amounts of pure iron oxide dusts, including magnetite, in general has been assessed by various federal, state, and local agencies responsible for dust and pollution standards and control. The conclusions have usually been that iron oxides are in the group of the least hazardous dusts and are called "nuisance dusts." The American Conference of Governmental Industrial Hygienists has recommended an upper limit per 8-hour shift of 10 mg/m^3 for workers. A more conservative criteria was adopted for the general public. A limit was set by the National Air Pollution Control Administration (NAPCA) at 80 μg/m^3 [*Air Quality Criteria for Particulate Matter* (Publication AP-49, NAPCA, Government Printing Office, Washington, D.C., 1969)]. The exposure of our subjects to a single inhalation of about 1 mg of magnetite is no greater than the equilibium retention level estimated to result from the NAPCA standard.
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Embryonic Rodent Brain Contains Estrogen Receptors

Abstract. *Estradiol-binding proteins with the properties of putative estrogen receptors are present in cytosol extracts of embryonic mouse hypothalamus and other brain regions. These embryonic estrogen receptors are adultlike in their high affinity and limited capacity for estradiol, sensitivity to diethylstilbestrol, ability to adhere to DNA, and behavior during DNA-cellulose affinity chromatography. As early as 4 days before birth, mouse hypothalamus has approximately 40 percent of the adult concentration of hypothalamic estrogen receptors with these properties. These observations raise the possibility that embryonic rodent brain has the biochemical potential to respond to sex hormones and that the critical period of brain sexual differentiation could be initiated prenatally.*

Sexual differentiation of rodent brain is influenced, in part, by steroid hormones acting during a "critical period" of brain development (*1, 2*). Although in mice and rats it is believed to occur during early postnatal development, several lines of evidence suggest that the critical period of brain sexual differentiation actually begins during late embryonic development (*1, 2*). For instance, perinatal administration of androgens and estrogens to rodents masculinizes and defeminizes both the genitalia and adult sexual behavior (*1*). Also, the in utero proximity of females to males (and, presumably, exposure to intrauterine androgens) correlates with the degree of androgenized genitalia and sexual and aggressive behavior exhibited by adult female mice and rats (*2*).

Since sex hormones play a key role in sexual differentiation, there is considerable effort to demonstrate putative receptor proteins for sex hormones in rodent brain during the critical period. Recently, several reports have demonstrated putative androgen and estrogen receptors in neonatal rodent brain (*3–8*). However, in the case of embryonic tissue, technical problems associated with the maternal circulation have prevented a similar analysis. In the absence of data concerning the existence of putative receptors in embryonic brain, understanding of the biochemical mechanisms that underlie the critical period is limited.

In the present study we report a new approach to the analysis of embryonic estrogen receptor which provides a more complete characterization of the estrogen receptor mechanism in mouse brain throughout its critical period. For the purposes of detection and subsequent analysis, we require that putative embryonic estrogen receptors from both hypothalamus and "brain" (whole brain minus hypothalamus) bind [^{3}H]estradiol and adhere to DNA-cellulose, thus distinguishing them from other estradiol-binding activities in embryonic brain extracts. We then ask whether these putative embryonic estrogen receptors are qualitatively similar to those of the adult in their affinity for estradiol, their sensitivity to diethylstilbestrol (DES), their DNA-cellulose elution characteristics, and their regional distribution within the brain.

To make these assessments of putative embryonic estrogen receptor, we developed a special protocol for DNA-cellulose affinity chromatography (legend to Fig. 1). Using this new approach, we found that cytosols of embryonic brain contain estradiol-binding macromolecules that adhere to DNA-cellulose (*5*). Under our conditions for DNA-cellulose affinity chromatography, cytosol estrogen receptors adhere to DNA-cellulose in the absence of estradiol (*9*), while estradiol-binding proteins not adhering to DNA as well as endogenous hormones are eliminated by washing (*3, 10*). Thus, by this procedure estrogen receptor can be labeled with [^{3}H]estradiol in the virtual absence of other known estradiol-binding proteins. This is important for quantitative analysis of embryonic estrogen receptor because embryonic cytosols contain high concentrations of both maternal estrogens and at least one estradiol-binding protein, the perinatal binding protein known as α-fetoprotein (AFP) (*11*). This is also true for neonatal cytosols, but to a lesser extent because these components decrease rapidly after parturition. Other receptor assays (*6, 8*), including conventional DNA-cellulose affinity chromatography (*3, 12*), are not as suitable for quantitative analysis since they utilize AFP-containing cytosols.

Cytosols of embryonic day 17 (E17) hypothalamus and "brain" (representing a total of 120 male and female embryos) were directly chromatographed on parallel DNA-cellulose columns, the columns were washed, and adhering macromolecules were then labeled with various concentrations of [^{3}H]estradiol. An estradiol-binding activity from E17 mouse hypothalamus (Fig. 1a) and "brain" (Fig. 1b) adhered to DNA-cellulose and reproducibly eluted with characteristics of estradiol receptor in a linear concentration gradient of NaCl. At all estradiol concentrations, a major estradiol-binding activity eluted with approximately 210 to 220 m*M* NaCl while a minor activity eluted with approximately 250 to 260 m*M* NaCl. These activities eluted from DNA-cellulose at the same salt concentrations as the 4*S* and 5*S* forms, respectively, of estrogen receptor from mouse uterine cytosols (*13*).

The putative estrogen receptor from both E17 hypothalamus and E17 "brain" appear to behave very similarly in that the major peak of estradiol-binding is positioned at 210 to 220 m*M* NaCl. At all embryonic and postnatal ages studied, we observe a slight qualitative difference in the overall elution patterns of hypothalamic versus "brain" receptor, although the data do not yet allow us to assess the significance of this subtle difference. Perhaps these patterns correlate with regional differences in the biological activity of these estrogen receptors. For example, estradiol induces an increase in progesterone receptor content in hypothalamus but not in other brain regions (*14*).

Comparison of the elution patterns obtained at several estradiol concentrations, as typified by Fig. 1, indicates that the DNA-adhering estradiol-binding activities in both E17 hypothalamus and "brain" saturate at estradiol concentrations between 3 and 8 n*M* (*15*). We find that putative embryonic estrogen receptors (from hypothalamus as well as "brain") at all ages tested (E16, E17, and E18) saturate in the same range of estradiol concentrations.

The data summarized in Fig. 1 also illustrate that macromolecular-bound [^{3}H]estradiol is fully competed by a 100-fold excess of nonradioactive DES. This behavior is typical of prepubertal estro-

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