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LUNG REACTION TO CHRONIC INHALATION OF
THREE CHRYSOTILES IN THE FISCHER 344 RAT. I. MORPHOMETRY

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ABSTRACT

LUNG REACTION TO CHRONIC INHALATION OF THREE TYPES OF CHRYSTILE ASBESTOS DURING THE LIFESPAN OF THE FISCHER 344 RAT

The quantitation of lung reaction to aerosolized chrysotile asbestos was determined by using morphometric techniques at the light and electron microscopic levels of magnification. Three different preparations of chrysotile were used to expose male and female Fischer 344 rats 7 hours per day, 5 days per week for 12 months. Randomly selected animals were examined following 3 and 12 months of exposure. Some animals exposed for 12 months were kept in filtered air for an additional 12 months before evaluation.

Fiber characterization of each aerosolized chrysotile preparation was done using light and scanning electron microscopy. Fiber length and fiber diameter measurements demonstrated unique size distribution characteristics for each aerosolized chrysotile preparation.

Exposure to each type of chrysotile caused different patterns of lung reaction. Lung reaction was most severe in the epithelial and interstitial compartments. Partial resolution of the epithelial reaction occurred during the postexposure period, while the interstitial reaction persisted. The differences in lung reaction to these three types of chrysotile are likely to be a result of differences in fiber length and in deposition of fibers within the lung parenchyma. Both short and long fibers elicit lung reaction. However, long fibers ($> 50 \mu\text{m}$) cause a more intense reaction than do short fib-

ers ($< 30 \mu\text{m}$). Fiber localization within the lung tissue compartments and ultrastructural changes which occurred following chrysotile inhalation were examined by light and transmission electron microscopy at each of the three time periods studied.

Because of the extensive period of time covered by this study, the effects of aging on morphological changes in the lung were examined over the lifespan of the Fischer 344 rat.

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CHAPTER 4

LUNG REACTION TO CHRONIC INHALATION OF THREE CHRYSOTILES IN THE FISCHER 344 RAT

I. MORPHOMETRY

INTRODUCTION

Lung injury caused by the inhalation of asbestos was first reported in 1907 in the autopsy findings of a cardroom worker in an asbestos factory. Extensive areas of this worker's lungs had become fibrotic and numerous "spicules of asbestos" were found both free and within the lung parenchyma (Murray, 1907). The term "asbestosis" was first used to identify this type of lung injury in 1927 (Cooke and McDonald). The induction of pulmonary fibrosis by inhalation of either the amphibole or serpentine class of asbestos dust in humans and animals is well documented (Vorwald et al., 1951; Wagner et al., 1974; Seal, 1980; Davis et al., 1980). Chrysotile, a member of the serpentine group, makes up 90% of the total asbestos production today. The use of chrysotile in insulation, ceiling tiles, brake linings, cement products, hairdryers and numerous other materials greatly enhances the number of people coming into contact with this type of asbestos. The biological effects of chrysotile have been studied using both natural and unnatural means of fiber exposure in laboratory animals. The fibrogenic and carcinogenic effects of chrysotile have been confirmed by inhalation (Vorwald et al., 1951; Wagner et al., 1974) and intratracheal and intrapleural injection of fibers (Suzuki, 1974; Wagner et al., 1973, 1980).

In vitro studies have also implicated chrysotile to be both cytotoxic (Sakabe et al., 1971; Allison, 1971; Beck et al., 1971; Richards et al., 1977) and hemolytic (Harrington et al., 1975; Desai et al., 1975).

The purpose of this study is to quantitate the degree and pattern of lung injury caused by the inhalation of three different chrysotile preparations. Three approaches are used: (1) grading (using a scoring system) of changes in the nonalveolar portions of the lung by light microscopy, (2) application of morphometric techniques to determine cell numbers, tissue volumes and surface areas in the parenchymal portions of the lungs by electron microscopy and (3) qualitative evaluation of structural changes and fiber localization in the lungs by both light and electron microscopy. This chapter deals with the second approach, while part II (Chapter 5) will cover the first and third.

N_o P Quantitative measurements of alveolar tissue structure in the lungs of rats following increasing periods of fiber inhalation and after a post-exposure period of time allows us to examine the acute and chronic effects of fiber inhalation for three types of chrysotile. Employment of morphometric techniques provides an objective means of comparing the relative fibrogenicity and degree of lung injury caused by each type of aerosolized chrysotile. In addition, we can identify those tissue compartments in the alveolar regions of the lung which are most affected by fiber exposure and the changes which occur in each alveolar tissue compartment following different periods of fiber inhalation.

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MATERIALS AND METHODS

Animals

Three hundred thirty (330) age-matched specific pathogen-free male and female Fischer 344 rats (weighing 125-150 grams) were acquired from Charles River Laboratory, Inc. Ten rats were randomly selected and killed. Total body necropsies found these animals to be free of (1) external and internal parasites, (2) salmonella in the feces, (3) respiratory pathogens, e.g., *Mycoplasma pulmonis*, *Streptococcus pneumoniae* and *Corynebacterium kutscheri*, (4) murine viruses as determined by serology and (5) disease of all major organs. Forty animals of each sex from the remaining 320 animals were assigned by random selection to either a control group or one of the three exposure groups.

Chrysotile Preparations

The three asbestos samples used in the inhalation studies were UICC B chrysotile, Jeffrey Mine chrysotile and Coalinga Mine chrysotile. A description of the origin and physical characteristics of each chrysotile sample has been reported (Pinkerton et al., 1982).

UICC B Chrysotile Reference Sample - This preparation, a grade 4 chrysotile (Timbrell et al., 1968A) was obtained from eight different Canadian chrysotile mines and mixed according to the proportional production of each mine during the year of 1950. The approval of this preparation as a reference standard was made in 1966 by the Union Internationale Contre Cancer (UICC) as an attempt to standardize asbestos materials used in experimental research. After aerosolization in our chambers, this preparation had fibers longer than 100 μ m in length, but 93% of all fibers were less than 10 μ m in length, and no fiber, regardless of length, exceeded 2 μ m in diameter (Pinkerton et al., 1982).

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Jeffrey Mine Chrysotile - Identified as Plastibest 20 by Johns Manville, this form of chrysotile is a grade 4 asbestos used by the plastics industry. The mine is located in Asbestos, Quebec, Canada. This sample was originally purchased by the National Institute of Environmental Health Sciences (NIEHS) for oral ingestion studies. The material was prepared by roller milling and air classification using standard industrial techniques, followed by passing the material through a Hurricane Pulverizer three times to open fiber bundles. In the aerosolized state, fibers longer than 100 μm were present, 92% of all fibers were less than 10 μm in length and no fibers exceeded 2 μm in diameter, regardless of length (Pinkerton, et al., 1982).

Coalinga Mine Chrysotile - Identified as COF-25 by Union Carbide, this unique form of chrysotile was mined from the New Idria serpentinite mass located in the Diablo range in California. The deposit is unusual in that the fibers are randomly oriented as a mat, rather than as parallel fibers running in veins and the mining process is done with bulldozers. The deposit is almost pure chrysotile. The fibers are short in length and are not of spinning grade. The processing of this material was done by roller milling and water classification. In the aerosolized state, fibers greater than 100 μm were present, 66% of all fibers were less than 10 μm in length and many fibers exceeded 2 μm in diameter. In general, as the length of the fiber increased, the diameter of the fiber also increased (Pinkerton et al., 1982).

For fibers longer than 5 μm in length and under 3 μm in diameter, it was found by light microscopy that the Jeffrey Mine chrysotile contained a significantly greater fraction of fibers in the 40-50 μm , 50-100 μm and >100 μm length intervals than that found in the UICC B or Coalinga chrysotile samples in the aerosolized state. Fiber analysis by scanning electron microscopy also revealed that of all aerosolized fibers longer than 10 μm in

length, the percent with a diameter less than $0.6 \mu\text{m}$ was 92.9% for Jeffrey chrysotile, 85.3% for UICC B chrysotile and 12.0% for Coalinga chrysotile (Pinkerton et al., 1982).

Exposure Conditions

Four 5.0 m^3 stainless steel chambers were used to house in each 40 male and 40 female rats in hanging $17 \times 33 \times 15 \text{ cm}$ wire cages, two animals per cage. The animals were continuously maintained in the chambers. Three chambers were used to expose animals to one of the three chrysotile preparations by fiber aerosolization using a modified Timbrell generator (Timbrell et al., 1968) which was operated 7 hours/day, 5 days/week for 12 months. The controls breathed filtered air and were not exposed to chrysotile.

The total chamber dust mass concentration was monitored daily to maintain a constant dust concentration throughout the duration of the exposure periods. These measurements were made by sampling 100 liters of air from the chamber at a flowrate of 10 liters/minute and collecting the airborne fibers and particles on a 25 mm glass fiber filter held in a Gelman filter holder. After the samples were collected, the filters were removed and weighed and the dust concentration in each exposure chamber was expressed in mg/m^3 . Size selective dust samples were also taken daily using a Casella type 113A Gravimetric Dust Sampler to determine the "respirable" dust concentration in each chamber. With the Casella Sampler placed inside the exposure chamber, samples were collected for 6 hours at a flowrate of 2.5 liters/minute. A multihorizontal elutriator system within the instrument was calibrated to prevent particles whose equivalent aerodynamic diameter exceeded $7.1 \mu\text{m}$ from collecting on the filter. The filter was weighed at the end of the sampling period to determine the "respirable" mass

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concentration in the exposure chamber. A summary of the daily means for the total and respirable dust concentrations are given in Table 1.

Animal Sacrifice and Tissue Preparation

Four animals of each sex were randomly selected from each exposure chamber and the control chamber after 3 months of fiber inhalation and again after 12 months of fiber inhalation. Full body necropsies were performed on these animals and will be reported elsewhere. The remaining animals were removed from the exposure chambers following 12 months of fiber inhalation and maintained behind a limited access barrier. Twelve months following the end of dusting, four animals of each sex were randomly selected from the surviving animals in each exposure group and the control group. The percent of survival was the following for each group: 61% in animals exposed to Coalinga Mine chrysotile, 53% in animals exposed to UICC B chrysotile, 52% in animals exposed to Jeffrey Mine chrysotile and 64% in animals which served as controls. The necropsy findings and lifespan characteristics of these randomly selected rats and the surviving animals will be reported elsewhere.

The rats used in this study were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg) and the trachea cannulated. The abdomen was opened, both hemidiaphragms punctured to collapse the lungs and fixative was instilled intratracheally via the tracheal cannula at a pressure of 20 cm of water. The size of the cannula and the tubing connecting it to the fixative were adjusted to give a flowrate equal to 115 ml/minute when the cannula is not inserted into the trachea. The fixative was 2% glutaraldehyde buffered to 7.4 using Na cacodylate and having a total osmolality of 350. After a minimum of 30 minutes fixation the lungs were removed from the chest cavity and the lung volume was measured by water displacement

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(Scherle, 1970). This technique of lung fixation has been shown to fix the lungs at approximately 75% of their physiologic TLC (Hayatdavoudi et al., 1981).

Light Microscopy

The right cardiac lobe from each animal was embedded in paraffin. Serial sections 10 μm thick were made through the entire lobe. Every fortieth section was placed on a glass slide and stained with hematoxylin and eosin. These were used to determine the parenchymal fraction of the lung as described in the morphometric analysis section of this paper.

Electron Microscopy

Four sites from the peripheral region of the left lung in each animal were randomly selected. These sites were chosen from the ventral and dorsal portions of the cephalic and caudal regions of the lung. The tissue samples from each site were washed in cacodylate buffer, dehydrated in a graded series of alcohol, transferred to increasing concentrations of propylene oxide, and embedded in Epon. Sections 0.5 μm thick were cut and stained with toluidine blue to verify the presence of alveolar tissue. Parenchymal tissue was considered to include alveoli and alveolar ducts. Airways with ciliated epithelium and blood vessels with a diameter greater than 25 μm were considered to be nonparenchymal tissue. Sections containing parenchymal tissue were cut using a diamond knife, placed on copper 200 mesh grids and poststained with uranyl acetate and lead citrate.

Morphometric Analysis

The morphometric procedures followed in this study have been well described by Weibel (1979), Crapo and Greeley (1978), Crapo, et al. (1978) and Woody, et al. (1980). The fraction of the total lung volume which

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constitutes the parenchymal region was determined by point counting each of the step sections taken through the right middle lobe of each animal. These paraffin step sections were directly placed in a Durst photographic enlarger and projected, at a constant magnification, onto 11 x 14" photographic paper. The resulting print was a negative image of the section. These prints were placed under a test lattice containing 224 points and the distribution of these points falling on either parenchyma or non-parenchyma were determined. Parenchymal areas included alveoli and alveolar ducts. Nonparenchymal areas included blood vessels greater than 25 μ m in diameter, interlobular connective tissue, bronchioles and bronchi. The above technique was found to be faster than doing the same analysis using an automatic sampling stage light microscope.

Fifteen random electron micrographs were taken of each of four sections prepared from peripheral lung sites for each animal and printed on 11 x 14" paper at a final magnification of 8500. Morphometric analysis of these points were done as described previously (Crapo, et al., 1978).

Numerical density of lung cells (N_V) was determined by using the formula: $N_V = N_A/D$, where N_A is the number of nuclear profiles per unit area on a thin section and D is the mean caliper diameter of the nuclei (Greeley et al., 1978; Woody et al., 1980). The nuclear profiles found in 100 consecutive grid squares (25 grids per section for 4 sections per animal) totally covered by the tissue section were counted. Grid squares containing non-parenchymal portions of the lung were omitted. Each nuclear profile was classified according to cell type using the unbiased counting rule described by Gunderson (1977). It was found that the area of the grid square on any single grid varied less than 2%, but that variation did occur between grids. Therefore, one of the grid spaces for each section was photographed at 500 X and its area determined by digitization. The total area counted for nuclear

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profiles for each grid was calculated as the number of grid squares counted times the area of a single grid space for that grid. The mean caliper diameter ($\bar{D}$) of each cell type was determined by taking light micrographs of serial nuclear profiles 0.5 μm thick through the entire nuclei of randomly selected cells from each of the major alveolar cell types. Each micrograph was enlarged to 4000 times actual size and the outline of all profiles making up one nucleus was traced using a Hewlett Packard 9874A Digitizer and serially reconstructed in three dimensions with a Hewlett Packard 9845A Computer. Computer analysis of this data, as described by Woody et al. (1980), was used to determine the mean caliper diameter for each reconstructed nucleus.

Lung Ashing and Silica Determination

The cranial, caudal and accessory lobes of the right lung from animals exposed to the three chrysotile preparations for 3 and 12 months were dried to a constant weight at 105°C and then ashed at a temperature of 460°C in a platinum crucible. The ash was mixed with anhydrous sodium carbonate and heated to 1000°C. Distilled water was added to the fused mass and the solution was decanted several hours later in H_2SO_4 . HCl and H_2SO_4 were used to remove any remaining material from the crucible. This mixture was allowed to evaporate and then distilled water was added to bring the total volume to 50 ml. This solution was then mixed with the following compounds: ammonium molybdate, oxalic acid-sulfuric acid, and ammonium ferrous sulfate. The mixing of these compounds resulted in reduction of silicomolybdic acid to a blue colored solution which was colorimetrically analyzed with a Technicon Autoanalyzer. Benusil, a silica compound, was used as the standard to determine the amount of silica present in solution (King et al., 1955).

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Statistical Analysis

All statistical comparisons were made by using a one-way analysis of variance followed by Duncan's multiple comparison test to determine the significant changes occurring between and within the various control and treatment groups.

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RESULTS

Body Weight and Fixed Lung Volumes

The mean body weight and fixed lung volumes are given in Table 2. The mean body weight for female rats exposed to each type of chrysotile was decreased following 3 months exposure compared to control. With continued exposure to chrysotile, the mean body weight was not significantly different from the age-matched control values. The mean body weight of the male rats was relatively unchanged from that of the controls with the exception of a decreased body weight in males exposed for 12 months to Jeffery chrysotile and an increased body weight in males one year following the end of exposure to Coalinga chrysotile.

The mean fixed lung volumes were significantly increased compared to control in male rats following 3 months exposure to all fibers. The fixed lung volume remained significantly elevated in the male rats exposed to Jeffrey chrysotile following 12 months exposure to 21% greater than control and one year after the end of exposure to 36% greater than the age-matched control values. Female rats exposed to Jeffrey chrysotile for 12 months and UICC B exposed females kept an additional year in filtered air also had a mean lung volume significantly greater than the age-matched controls. Only females exposed for 12 months to either Coalinga or UICC B chrysotile had a significantly smaller mean lung volume than the control value.

Light Microscopy

The mean parenchymal fraction found for the lungs of each group of animals ranged from 0.79 to 0.82. This range in values was not statistically significant, but was a result of neoplastic changes occurring in the parenchymal portions of the lung. Most lung tumors were found peripherally one year following the end of fiber exposure. The mean percent of the lung

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with tumor was 0.1% in the animals exposed to Coalinga chrysotile, 0.7% in the animals exposed to UICC B chrysotile and 1.4% in the animals exposed to Jeffrey chrysotile. These tumors consisted of adenomas, squamous cell carcinomas and bronchiolar metaplasia. The exclusion of these neoplastically altered regions of the parenchyma from the parenchymal fraction of the lung did not significantly affect the morphometric results of this study.

Electron Microscopy

The volumes and surface areas of the three major components of the parenchymal portion of the lungs, air, capillary blood and tissue are given in Table 3. Male rats exposed to Jeffrey chrysotile were the only group to have a total alveolar airspace volume significantly greater than controls at all three time periods, with 13%, 17% and 29% increases respectively over the age-matched controls. The pulmonary capillary blood volume was not significantly changed from controls in any of the exposed groups. Total alveolar tissue volume increased in male rats following 3 and 12 months exposure to each chrysotile preparation. One year following the end of fiber exposure, the alveolar tissue volume in male rats exposed to UICC B showed a progressive increase which was significantly greater than that occurring in controls. Female rats showed similar trends toward greater parenchymal tissue volume after each chrysotile exposure but these changes did not reach a statistical level of significance except for those exposed to the Jeffrey chrysotile which had a 154% increase in tissue volume after 12 months exposure and an 84% increase one year following 12 months exposure.

The absolute surface area of the alveolar epithelium and the capillary endothelial lumen demonstrated no substantial changes between the controls and treatment groups for both males and females. The capillary endothelial

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surface area in females one year following the end of exposure to UICC B chrysotile was significantly decreased from control. However, a similar trend was not seen in the corresponding males exposed to UICC B chrysotile. Therefore, we have not attributed any biologic significance to these differences.

The alveolar tissue volume of the lung is subdivided in figure 1 into its three basic components; epithelium, interstitium and endothelium. The volume of endothelium was unchanged from control values for each exposure group except for a 49% and 57% increase respectively in the male and female animals exposed to Jeffrey chrysotile for 12 months and then to filtered air for 12 months. Exposure to all three types of chrysotile for 3 months resulted in an increased alveolar epithelial volume in male and female rats. Following 12 months exposure, the epithelial volume was also significantly increased for all three treatment groups compared to the age-matched controls. The increase in epithelial volume was greatest in the animals exposed to Jeffrey chrysotile for 12 months, being 175% and 201% in males and females respectively. Following 12 months exposure to UICC B chrysotile, the alveolar epithelial volume in the male and female rats was 88% and 75% greater than control respectively. Only the animals exposed to Coalinga chrysotile showed a decrease in the epithelial volume from 3 months of exposure to 12 months of exposure with a 20% decrease in the males and a 19% decrease in the females, but the absolute epithelial volume was still significantly greater than control. One year following the end of 12 months fiber exposure, the alveolar epithelial volume in all exposure groups decreased for both male and female animals.

The alveolar interstitial volume also increased in the lungs of male and female rats following 3 months exposure for all three chrysotile samples. Following 12 months exposure, the interstitial volume in males and

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females dusted with Jeffrey chrysotile increased 146% and 157% of the respective control values. The alveolar interstitial volume in the lungs of male rats exposed to Coalinga and UICC B chrysotile for 12 months had also significantly increased by 53% and 50% of the age-matched control value respectively. One year following the end of 12 months exposure, the interstitial volume in the lungs of male and female rats exposed to Coalinga and Jeffrey chrysotile had increased by an average of 12% from the interstitial volume recorded at the end of fiber exposure. In contrast, the alveolar interstitial volume in the lungs of the age-matched male and female controls increased by an average of 24% during the same time period. Only the alveolar interstitial volume in male rats exposed to UICC B chrysotile for 12 months increased significantly during the postexposure year, by 83% from the value seen immediately following the end of 12 months exposure.

The changes occurring in total volume of alveolar type I and alveolar type II epithelium are given in Table 4. After 3 months exposure, total alveolar type II cell volume had more than doubled in the lungs of male and female rats from all three treatment groups. At the end of 12 months exposure, the type II epithelial volume continued to increase in males and females exposed to UICC B chrysotile (233% and 250% of control) and Jeffrey chrysotile (420% and 395% of control). The alveolar type I cell volume was also significantly increased in males following 12 months of exposure to each of the fiber preparations. One year following the end of fiber exposure, the total volume of alveolar type II cells had decreased for both male and female rats in all three treatment groups, although male rats exposed to UICC B chrysotile and female rats exposed to Jeffrey chrysotile had a significantly greater type II epithelial volume than that of the respective controls. During the postexposure year, the volume of the type I epithelium remained relatively unchanged.

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Table 5 divides the alveolar interstitium into two parts; the cellular component and the noncellular or matrix component. After 3 months exposure, the total volume of cells in the interstitium increased in the lungs of all treatment groups for both sexes. Following 12 months exposure, the only groups to maintain a significantly increased interstitial cell volume compared to the age-matched controls were the male and female rats exposed to Jeffrey chrysotile. One year after the end of exposure, males and females exposed to Jeffrey chrysotile and males exposed to UICC B chrysotile had significantly greater interstitial cell volumes compared to controls.

The interstitial matrix volume had begun to increase after 3 months exposure in both males and females from all three exposure groups. After 12 months exposure, a marked increase in the interstitial matrix volume had occurred in the male animals from each of the treatment groups. Compared to the control value, the interstitial matrix volume was 60% greater in male rats exposed to Coalinga chrysotile, 54% greater in males exposed to UICC B chrysotile and 143% greater in male rats exposed to Jeffrey chrysotile. The total volume of the interstitial matrix continued to increase during the postexposure period in control and treatment groups of both sexes. A unique change in the interstitial matrix volume during this postexposure period was seen in the lungs of rats which had been exposed to UICC B chrysotile. While the controls and groups exposed to Coalinga and Jeffrey chrysotile showed a gradual increase in the total interstitial matrix volume of the lungs, male rats and female rats to a lesser degree had a significant increase in the volume of the interstitial matrix volume during the post-exposure year. The increase in matrix volume in the males was a 4 to 5 fold greater increase than seen in the corresponding controls or the animals which had been exposed to Coalinga or Jeffrey chrysotile.

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The computerized serial reconstruction of nuclei to determine the mean nuclear diameter of the major alveolar cell types in the rat lung was done in four rats from the controls age-matched to the animals exposed to chrysotile for 12 months. Two controls of each sex were used and three rats which had been exposed for 12 months to Jeffrey chrysotile (2 males and 1 female). The animals exposed to Jeffrey chrysotile for 12 months were selected because they demonstrated the most dramatic lung reaction of all exposure groups. If reaction of the lung cells to asbestos caused a change in the mean nuclear diameter of each cell type, animals exposed for 12 months to Jeffrey chrysotile would be most likely to demonstrate these changes most clearly. The results of this study are given in Table 6. A jackknifed weighted mean for each cell type was determined (Greeley, et al., 1978) and values were compared between males and females, and between exposed animals and controls. It was found that there was no statistically significant difference between sexes or between controls and animals exposed to Jeffrey chrysotile for alveolar type I, alveolar type II, interstitial or macrophage nuclei. A difference was noted for the endothelial mean nuclear diameter between the exposed group and controls. It was decided to pool the data for each alveolar cell type to obtain an overall mean nuclear diameter for each class of cells. These values were used to calculate the cell density of each alveolar cell type. The mean nuclear diameter found for rats exposed to Jeffrey chrysotile for 12 months was used to calculate the endothelial cell number for that specific exposure group and time period. The endothelial cell number calculated for all other exposure groups and time periods used a pooled endothelial mean nuclear diameter. It should be noted that the use of either endothelial mean nuclear diameter does not cause significance changes in endothelial cell number that would have been created or lost by the use of one value over the other.

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Changes in alveolar cell numbers are given in Table 7. The total number of cells which make up the alveolar region of the lungs was most dramatically increased in male and female rats exposed to Jeffrey chrysotile for 12 months. In males the total number of cells had more than doubled, while in females the total cell number had increased to 166% of the corresponding control value. The three alveolar cell types showing the greatest changes in numbers and responsible for most of the increases in total cell number during and following exposure to the three types of chrysotile were alveolar type II cells, interstitial cells and alveolar macrophages.

A significant increase in alveolar type I cell number compared to controls occurred only in the lungs of animals exposed to Jeffrey Mine chrysotile (Table 7). Alveolar type II cell number was significantly increased in male rats after 3 months of exposure, 12 months of exposure and one year following the end of exposure for all three treatment groups (Table 7). The greatest type II cell proliferation was seen in the lungs of male rats exposed to Jeffrey chrysotile for 12 months (a 279% increase in comparison to the age-matched controls). One year following the end of exposure to Jeffrey fibers, the type II cell number in males decreased by 44%, but was still more than double the type II cell number seen in controls. In general, the pattern of alveolar type II cell proliferation in female rats exposed to the three chrysotiles was similar to that seen in the males. In animals exposed to Jeffrey chrysotile from 3 months to 12 months, females had an 84% increase in the number of alveolar type II cells while males had a 73% increase in type II cell number. Type II cell number was the highest in males and females exposed for 12 months to Jeffrey fibers compared to other treatment groups of the same sex and time periods. Figure 2 illustrates the percent change in the number of alveolar type I and alveolar type II cells in pooled male and female rats compared to the age-matched

controls. Figure 2 graphically illustrates the more pronounced changes which occurred in alveolar type II cell number compared to alveolar type I cell number in all exposure groups.

The number of interstitial cells in the alveolar region of the lung for both male and female rats was increased after 3 months exposure to all three chrysotile preparations from 22% to 70% above that of the controls. For animals exposed to Jeffrey chrysotile, the number of interstitial cells continued to increase after 12 months exposure, while animals exposed to Coalinga and UICC B chrysotile showed a decrease in the total number of alveolar interstitial cells during this time period in both sexes. One year after the end of exposure, male and female rats which had been exposed to UICC B and Jeffrey chrysotile demonstrated the highest numbers of interstitial cells compared to the controls ranging from 46% to 144% of control. This represented a significant increase over control with the exception of the male rats exposed to UICC B fibers. Figure 3 illustrates percent changes in the major components of the interstitium in pooled male and female rats. These are changes in cell number, changes in cell volume and changes in the matrix volume.

The total number of alveolar macrophages in the airspaces of the lung was increased following 3 months of exposure to the three different types of chrysotile (Table 7, Figure 4). After 12 months exposure, the number of macrophages was still at least doubled compared to control in all treatment groups for both sexes. Alveolar macrophage number was greatest in males and females exposed for 12 months to Jeffrey fibers with a 371% and 471% increase over the number seen in the corresponding controls. One year following the end of fiber exposure, only the males which had been exposed to Jeffrey chrysotile had a significantly increased number of alveolar macrophages compared to the controls. Figure illustrates the percent

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changes in the numbers of alveolar macrophages for pooled male and female rats.

Capillary endothelial cells in control animals comprised approximately one-half of the total number of cells in the alveolar region of the lungs. The total number of endothelial cells in animals exposed to Coalinga and UICC 8 remained relatively unchanged from that of the age-matched controls during the entire period of study (Table 7). In contrast, male and female rats exposed to Jeffrey chrysotile had a significantly increased capillary endothelial cell number following 12 months exposure which remained significantly increased one year after the end of fiber inhalation.

The harmonic mean tissue thickness of the air-blood barrier is given in Table 9. With the exception of male rats exposed for 3 months to Coalinga and UICC 8 chrysotile, all exposure groups for both sexes demonstrated a significantly increased harmonic mean thickness of the air-to-blood tissue barrier during and following fiber inhalation. Animals exposed to Jeffrey chrysotile demonstrated the greatest increase in the harmonic mean tissue thickness during the period between 3 to 12 months fiber inhalation with a 40% and 56% increase for males and females respectively.

The arithmetic mean thickness of the air-to-blood tissue barrier is illustrated in Figure 5. This thickness has been subdivided into the arithmetic mean thickness of the epithelium, interstitium and endothelium. The thickness of the alveolar epithelium was significantly increased at every time point studied for all three types of chrysotile. However, the thickness of the epithelium decreased by 23% to 78% in all groups during the postexposure period. The thickness of the interstitium was also significantly increased at almost every time point during and following exposure to each type of chrysotile. The arithmetic mean thickness of the interstitium increased by 60% in animals exposed to Jeffrey chrysotile for 12 months

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compared to those exposed for 3 months. The interstitial thickness in animals exposed to Coalinga and UICC B chrysotile increased by 3% and 23% respectively during the same time period. During the postexposure period, the interstitial thickness in animals which had been exposed to UICC B chrysotile increased by an additional 25%, while no increase was seen in animals dusted with the other two chrysotiles. The arithmetic mean thickness of the endothelium was significantly increased in animals exposed to Jeffrey chrysotile at each of the three time periods.

Lung Ashing and Silica Determination

The results of the lung ashing are given in Table 10. All treatment groups following 3 and 12 months of fiber inhalation had significantly greater amounts of silica in the lungs compared to the controls. The silica content of the lungs following 3 months fiber exposure was similar for the three treatment groups. Only the animals exposed to Jeffrey Mine chrysotile had a statistically significant increase in silica content of the lungs between 3 months and 12 months of exposure.

DISCUSSION

The degree of pulmonary damage brought about by the inhalation of asbestos has been postulated to be due to either the chemical composition of the fibers, the physical state of the fibers, the amount of fibers deposited in the lungs or some combination of these factors (Wagner, 1965; Seaton, 1975). Although animal experiments can closely control asbestos exposure conditions, the measurement of the relative fibrogenicity of different types of asbestos has been largely subjective and inconclusive, while the relative carcinogenicity of different types of asbestos has been more definitive in that tumors were or were not produced (Wagner et al., 1974). Other factors which are likely to contribute to the amount of lung fibrosis produced by different types of asbestos are the degree of fiber penetration into the lungs and the pattern of fiber deposition in the lung. Both fiber penetration and deposition are related to the size and aerodynamic properties of the fiber (Timbrell, 1965, 1970, 1973).

New insights into the pathogenicity and fibrogenicity of inhaled chrysotile can be obtained by correlating the physical characteristics of different aerosolized chrysotile preparations and the degree of fiber deposition achieved with quantitative measurements of lung structure. Inhalation of all three chrysotiles included in this study resulted in both an epithelial and interstitial reaction in the lung parenchyma. An increase in the volume and in the number of alveolar type II cells and interstitial cells occurred following 3 months of fiber inhalation. With progressive exposure, the epithelial and interstitial reaction was most pronounced in those animals inhaling Jeffrey chrysotile.

Following three months exposure to aerosolized fibers, the amount of lung injury in each of the three treatment groups was similar. These

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results suggest that inhalation of both short and long fibers can acutely lead to epithelial and interstitial injury and to cell proliferation in these alveolar compartments of the lung. The shorter and thinner fibers may be able to penetrate more efficiently than longer fibers. Shorter fibers may also be more efficiently cleared from the lungs since alveolar and interstitial macrophages can engulf the entire fiber and bring about its removal through either digestion or clearance of the cell from the lung via the lymphatic and or mucociliary escalator.

Differences in the patterns of lung reaction in rats with exposure to each type of chrysotile may be due to differences in the physical characteristics of each chrysotile preparation in the aerosolized state. Coalinga Mine chrysotile, which contained fibers up to 100 μm in length in the aerosolized state, had few fibers greater than 30 μm in length that were also less than 0.6 μm in diameter. Previous studies have suggested that fibers with a diameter greater than 0.5 μm are unlikely to reach the alveolar airspaces of the lung. Therefore, only the shorter fibers (30 μm in length) in the aerosolized Coalinga Mine chrysotile are likely to be respirable. This makes this chrysotile preparation a "relatively short" fiber preparation in comparison to the other two chrysotiles studied. Correlation of the physical characteristics of each aerosolized fiber preparation to the patterns of lung injury following exposure suggest that short fibers or fibers less than 30 μm in length are less toxic than fibers of greater length. However, even short fibers less than 30 μm in length can cause lung reaction, particularly in the alveolar epithelium and interstitium.

Animals exposed to Jeffrey chrysotile respired a greater portion of fibers which had both a long length (40 to >100 μm) and a small diameter compared to animals exposed to the other two chrysotile preparations. Animals exposed to Jeffrey fibers demonstrated the greatest degree of lung

injury during fiber inhalation compared to animals exposed to the other two chrysotiles. These results suggest that fibers in the 40 to > 100 μm length range are less in number, but are the most toxic fiber in the lung parenchyma.

Information concerning the amount of chrysotile reaching and being retained in the lungs may be estimated from the amount of silica remaining in the lungs of dusted rats. Animals exposed to Coalinga chrysotile had a mean of 610 μgm of silica in the lungs following 3 months of fiber inhalation which was unchanged following an additional 9 months of fiber exposure. Animals exposed to UICC B chrysotile showed a small, but not statistically significant increase in the amount of silica in the lungs from 3 to 12 months of exposure. Animals exposed to Jeffrey chrysotile had a 52% increase in silica content in the lungs from 3 to 12 months of exposure but this was a proportionately less accumulation of silica than occurred during the first 3 months of exposure (Table 9).

The patterns of lung reaction with inhalation of the three chrysotiles correspond with the silica content found in the lungs. Animals exposed to Coalinga and UICC B chrysotile showed relatively no changes in the epithelial or interstitial tissue compartments from 3 to 12 months of exposure, while animals exposed to Jeffrey chrysotile showed dramatic reactions in both the epithelium and the interstitium.

Based on the physical characteristics of the aerosolized fibers and the amount of silica retained in the lungs, it appears that animals exposed to Coalinga chrysotile have reached a state of equilibrium in the fiber load of the lungs in which fibers were cleared at approximately the same rate in which they were deposited. On the other hand, animals exposed to Jeffrey chrysotile had not yet reached equilibrium following 3 months of fiber inhalation. It is possible that the gradual deposition of long fibers in the

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lungs of animals exposed to UICC B or Jeffrey chrysotile is responsible for the increasing silica load being retained in the lungs of animals given these exposures for a total of 12 months. Long fibers which cannot be taken up by one cell and removed from the lungs may eventually become trapped within the interstitial matrix of the alveolar septa and never be removed. Although the means of fiber accumulation cannot be determined by the measurement of silica content in the lungs, the gradual accumulation of a relatively small number of long fibers in the lung parenchyma could lead to significant increases in the silica content of lungs of animals exposed to aerosolized Jeffrey chrysotile for 12 months.

One year following the end of fiber exposure, the epithelial reaction in the lungs had, in part, resolved with decreases in the volume and in the number of alveolar type II cells. The resolution of the epithelial reactions was most notable in those animals exposed to Jeffrey chrysotile. One year following the end of fiber exposure, the interstitial reaction had not significantly resolved. However, increases in the volume of the interstitial matrix were seen in each exposure group. The interstitial cell number in animals exposed to each chrysotile preparation did decrease during the postexposure year. A surprising finding was the dramatic increase in the volume of the interstitial matrix in the lungs of animals which had been exposed to UICC B chrysotile (Figure 3).

The pattern of these changes occurring during the postexposure year in each exposure group add support to the importance of fiber length and fiber load in causing lung reaction. Animals which had been exposed to Coalinga chrysotile had relatively no changes in lung structure from that seen after 3 months of fiber inhalation with the exception of a significant decrease in the volume of the alveolar type II epithelium and a gradual increase in the volume of the interstitial matrix in the alveolar regions of the lung. In

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animals which had been exposed to Jeffrey chrysotile, the lungs also demonstrated a decrease in the volume of the alveolar type II epithelium and a gradual increase in the volume of the interstitial matrix. Only in animals which had been exposed to UICC B chrysotile did the volume of the interstitial matrix continue to increase significantly during the postexposure year. This increase in the interstitial matrix volume in the lungs of rats exposed to UICC B chrysotile may reflect the presence of long fibers in the lungs which were not cleared and continued to be a stimulus for the continued deposition of collagen within the interstitium. The analysis of fiber size distribution for aerosolized UICC B chrysotile demonstrated the presence of long fibers with a diameter sufficiently small to allow penetration to the alveolar airspaces of the lung, but with sufficient length to create immense problems in clearance. This same type of fiber was also present in the aerosolized Jeffrey chrysotile, only in proportionately greater numbers. It is feasible that the presence of fewer long fibers in the lungs of animals exposed to UICC B chrysotile resulted in a slower rate of lung fibrosis which continued to progress during the postexposure period. The greater exposure to very long fibers ($> 50 \mu\text{m}$) in the animals exposed to Jeffrey Mine chrysotile stimulated a much more intense initial inflammatory reaction which encased many of these fibers in layers of noncellular, collagenous interstitial matrix thereby reducing their inflammation inducing potential. This would have allowed the inflammatory reaction in these animals to have decreased in intensity during the postexposure year in air while the reaction in the UICC B chrysotile exposed animals progressed at an initially slower but steady pace. The presence of fewer long fibers in the lungs of rats exposed to UICC B chrysotile after 12 months compared to the corresponding rats exposed to Jeffrey chrysotile is indirectly supported by

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the significantly smaller amount of silica in the lungs of rats exposed to UICC B fibers compared to the animals exposed to Jeffrey fibers.

In summary, these different patterns of lung reaction are likely to be a result of differences in fiber length and in deposition of fibers within the distal portions of the lung parenchyma. Both short ($< 30 \mu\text{m}$) and long ($40 \mu\text{m}$ to $> 100 \mu\text{m}$) fibers are capable of eliciting a tissue response in the lungs which is most pronounced in the epithelial and interstitial compartments. Exposure to proportionately greater numbers of long fibers ($> 50 \mu\text{m}$) probably leads to the accumulation of a greater fiber load in the lungs than would occur with exposure to fibers less than $30 \mu\text{m}$ in length. Fibers less than $30 \mu\text{m}$ appear to reach an equilibrium in the lungs in terms of deposition and clearance. Fibers greater than $40 \mu\text{m}$ may be less efficiently cleared from the lungs and are likely to cause epithelial and interstitial reactions until they can be dissolved, cleared or isolated. The proportion of long fibers in the lungs correlates with the intensity of the tissue reaction. Exposure to large numbers of long fibers ($> 50 \mu\text{m}$) cause an intense tissue response within the epithelial and interstitial compartments. An exposure to smaller numbers of long fibers appears to cause a less intense, but more prolonged tissue reaction. The progressive tissue reaction seen in this situation may be due to the fact that the initial inflammatory response is not severe enough to totally encase the fibers and thereby remove them as a major source of irritation to the lung parenchyma. Chapter 5 will illustrate a number of these findings.

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TABLE 1

MEAN DAILY AND CUMULATIVE RESPIRABLE AND TOTAL CHAMBER DUST
CONCENTRATION OF EACH CHRYSOTILE BY MASS DETERMINATION

CHRYSOTILE	DAILY RESPIRABLE DUST CONCENTRATION FOR FIRST 3 MONTHS*	CUMULATIVE #	DAILY TOTAL DUST CONCENTRATION FOR FIRST 3 MONTHS*	CUMULATIVE #
Coalinga	3.29 $\pm$ 1.42	1451 mg-hrs	8.75 $\pm$ 2.28	3859 mg-hrs
UICC B	8.68 $\pm$ 1.97	3828 mg-hrs	11.15 $\pm$ 2.89	4917 mg-hrs
Jeffrey	9.06 $\pm$ 1.83	3995 mg-hrs	10.25 $\pm$ 2.33	4520 mg-hrs

CHRYSOTILE	DAILY RESPIRABLE DUST CONCENTRATION FOR 12 MONTHS*	CUMULATIVE #	DAILY TOTAL DUST CONCENTRATION FOR 12 MONTHS*	CUMULATIVE #
Coalinga	3.28 $\pm$ 0.83	5442 mg-hrs	7.76 $\pm$ 1.46	12874 mg-hrs
UICC B	8.32 $\pm$ 1.75	13919 mg-hrs	10.99 $\pm$ 2.11	18386 mg-hrs
Jeffrey	9.90 $\pm$ 1.63	16286 mg-hrs	11.36 $\pm$ 2.18	19687 mg-hrs

*Daily respirable and chamber dust concentrations are mean $\pm$ SD, n = 56-56 for 3 months, n = 235-239 for 12 months, values are expressed as mg/m³

#Cumulative measurements of respirable and chamber dust concentrations represent the respective mean concentrations times the number of hours the Timbrell generator was operating.

TABLE 2

MEAN BODY WEIGHT AND FIXED LUNG VOLUMES

MALES				
	CONTROL	COALINGA	UICC B	JEFFREY
Body weight (grams)				
Exposure times				
3 months	289±13	306±5	301±10	281±6
12 months	377±3	367±12	375±5	337±14*#
12 mo. + 12 mo. air	391±11	446±11*	405±6	379±9
Fixed lung volumes (cm ³)				
Exposure times				
3 months	8.60±.31	9.69±.30*	9.91±.32*	9.88±.25*
12 months	10.58±.09	9.44±.38	10.21±.12	12.76±.33*#
12 mo. + 12 mo. air	12.67±.74	11.70±.27	12.39±.17	17.24±1.35*#
FEMALES				
	CONTROL	COALINGA	UICC B	JEFFREY
Body weight (grams)				
Exposure times				
3 months	182±5	167±5*	164±3*	163±5*
12 months	218±8	205±7	205±6	198±6
12 mo. + 12 mo. air	298±7	286±13	284±12	271±10
Fixed lung volumes				
Exposure times				
3 months	7.48±.10	6.78±.24	7.06±.17	7.10±.33
12 months	7.80±.27	6.96±.06*	6.89±.11*	9.35±.06*#
12 mo. + 12 mo. air	9.39±.40	9.10±.13	9.03±.23	11.25±.48*#

All data are mean ± SEM, n = 4

*p < 0.05 for comparisons to the age-matched controls.

#p < 0.05 for comparisons to the group exposed to Jeffrey chrysotile to both of the other age-matched fiber exposure groups.

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TABLE 3
ABSOLUTE VOLUMES OF ALVEOLAR AIR SPACE, CAPILLARY LUMEN AND ALVEOLAR TISSUE
DURING AND FOLLOWING ASBESTOS INHALATION

Total volume (mm ³ /both lungs)	MALES			FEMALES		
	CONTROL	COALINGA	UICC B	JEFFREY	CONTROL	COALINGA
Air						
3 months	5905±195	6378±102	6823±213*	6668±318*	5248±168	4717±212
12 months	7544±81	6421±205	6913±37	8840±900*§	5570±163	4885±70
12 months + 12 mo air	9257±476§	8093±120§	8312±120	11986±1000*§	6989±341§	6562±180§
Capillary lumen						
3 months	641±57	724±119	608±53	545±56	452±97	373±102
12 months	573±28	576±99	580±49	363±96	435±65	362±47
12 months + 12 mo air	666±97	692±140	643±62	671±116§	343±47	419±65
Tissue						
3 months	422±46	746±30*	595±26*	657±57*	355±15	453±10
12 months	449±20	646±25*	676±55*	1007±67*§	309±22	414±30
12 months + 12 mo air	464±43	689±97	955±42*§	965±206*	370±22	390±12
						461±43
						680±58*#

Surface area (cm ² /both lungs)**	TOTAL SURFACE AREA OF THE ALVEOLAR EPITHELIUM AND CAPILLARY ENDOTHELIUM IN RAT LUNG DURING AND FOLLOWING ASBESTOS INHALATION					
	CONTROL	COALINGA	UICC B	JEFFREY	CONTROL	COALINGA
Alveolar epithelium						
3 months	4011±411	4567±312	4489±254	4165±216	3465±139	3109±240
12 months	4511±301	4111±261	4285±350	4485±301	3285±286	2919±150
12 months + 12 mo air	4695±415	4818±315	5397±341	4843±656	4078±38§	3448±322
Capillary endothelium						
3 months	3786±389	4136±438	4266±260	3866±198	3381±126	2887±190
12 months	4446±320	4132±392	4056±316	3474±542	3108±302	2703±128
12 months + 12 mo air	4491±484	4560±589	4970±272	3958±614	3569±163	3244±217
						2849±322
						3760±363

† All data mean ± SEM, n = 4.

* p < 0.05 for comparisons to the age-matched controls.

p < 0.05 for comparisons of the group exposed to Jeffrey chrysotile to both of the other age-matched fiber exposed groups.

§ p < 0.05 when comparing the 3 month group of a treatment group to the 12 month group of the same treatment or the 12 month treatment group to the 12 month chrysotile + 12 months air group of the same treatment.

** Surface area of the alveolar epithelium is the surface area of basement membrane under type I cells and the surface area of type II cells excluding the extra surface area contributed by microvilli; endothelial surface area is the luminal surface of the endothelial cells.

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TABLE 4

ABSOLUTE VOLUMES OF THE ALVEOLAR EPITHELIUM
DURING AND FOLLOWING ASBESTOS INHALATION[†]

	MALES			
	CONTROL	COALINGA	UICC B	JEFFREY
Total volume (mm ³ /both lungs)				
Type I epithelium				
3 months	81 ± 6	112 ± 3*	93 ± 8	95 ± 2
12 months	83 ± 3	113 ± 2*	113 ± 12*	155 ± 7*#§
12 mo + 12 mo air	84 ± 8	114 ± 15	116 ± 7	121 ± 22§
Type II epithelium				
3 months	36 ± 9	132 ± 14*	79 ± 12	123 ± 27*
12 months	30 ± 4	82 ± 4	100 ± 14*	156 ± 37*
12 mo + 12 mo air	28 ± 9	62 ± 15	96 ± 21*	73 ± 26§
FEMALES				
	CONTROL	COALINGA	UICC B	JEFFREY
Total volume (mm ³ /both lungs)				
Type I epithelium				
3 months	78 ± 7	80 ± 6	63 ± 5	79 ± 5
12 months	57 ± 7	64 ± 8	67 ± 8	133 ± 9*#§
12 mo + 12 mo air	66 ± 6	66 ± 8	79 ± 5	87 ± 8§
Type II epithelium				
3 months	25 ± 1	68 ± 6*	66 ± 7*	67 ± 5*
12 months	20 ± 3	56 ± 11#	70 ± 9*	99 ± 17*§
12 mo + 12 mo air	18 ± 5	29 ± 9§	41 ± 11§	70 ± 6*#§

† All data mean ± SEM, n = 4 for each group and time period.

* p < 0.05 when comparing the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA.

p < 0.05 when comparing groups exposed to Jeffrey chrysotile with all other asbestos-exposed groups of the same time period.

§ p < 0.05 when comparing a treatment group to its corresponding treatment group at the preceeding time point.

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TABLE 5
 ABSOLUTE VOLUMES OF THE ALVEOLAR INTERSTITIUM
 DURING AND FOLLOWING ASBESTOS INHALATION[†]

MALES				
	CONTROL	COALINGA	UICC B	JEFFREY
Total volume (mm ³ /both lungs)				
Cellular interstitium				
3 months	67 ± 5	124 ± 2*	104 ± 5*	131 ± 15*
12 months	57 ± 8	77 ± 3	80 ± 16	145 ± 16*
12 mo + 12 mo air	57 ± 7	81 ± 21	127 ± 2*	168 ± 54*
Noncellular interstitium				
3 months	126 ± 16	195 ± 22*	177 ± 3*	163 ± 13
12 months	149 ± 13	239 ± 15*	230 ± 25*	362 ± 27*#§
12 mo + 12 mo air	178 ± 20	287 ± 38	439 ± 25*§	422 ± 80*
FEMALES				
	CONTROL	COALINGA	UICC B	JEFFREY
Total volume (mm ³ /both lungs)				
Cellular interstitium				
3 months	59 ± 7	97 ± 9	72 ± 10	140 ± 24*
12 months	49 ± 4	47 ± 6§	62 ± 10	138 ± 11*#
12 mo + 12 mo air	56 ± 6	53 ± 9	46 ± 4	107 ± 16*#
Noncellular interstitium				
3 months	80 ± 10	82 ± 5	134 ± 11*	98 ± 5
12 months	109 ± 11	138 ± 9	146 ± 10	265 ± 21*#§
12 mo + 12 mo air	153 ± 8	168 ± 15	211 ± 28*§	282 ± 22*#

† All data mean ± SEM, n = 4 for each group and time period.

* p < 0.05 when comparing the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA.

p < 0.05 when comparing groups exposed to Jeffrey chrysotile with all other asbestos-exposed groups of the same time period.

§ p < 0.05 when comparing one treatment group to its corresponding treatment group of the preceeding time point.

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TABLE 6
MEAN CALIPER DIAMETER OF THE MAJOR ALVEOLAR CELL NUCLEI
IN CONTROL AND DUSTED FISCHER 344 RAT LUNGS (μm)

	ALVEOLAR TYPE I	ALVEOLAR TYPE II	INTERSTITIAL	ENDOTHELIAL	MACROPHAGE
CONTROL	8.23 ± 1.13	$7.18 \pm .64$	7.55 ± 1.06	$7.01 \pm .64$	$7.58 \pm .89$
n	25	29	35	47	19
(2 males + 2 females) age-matched to rats which were exposed to chrysotile for 12 months					
DUSTED	$8.28 \pm .74$	$7.40 \pm .57$	$7.16 \pm .98$	$7.52 \pm .82^*$	$7.56 \pm .70$
"	11	15	20	15	12
(2 males + 1 female) exposed for 12 months to Jeffrey chrysotile					
POOLED DATA	8.24 ± 1.02	$7.25 \pm .62$	7.41 ± 1.04	$7.13 \pm .49^{**}$	$7.58 \pm .81$
n	36	44	55	62	31

Jackknife weighted mean $\pm$ SD

* $p < 0.05$ for comparison of the endothelial mean nuclear diameter from control and dusted rats.

** The endothelial mean nuclear diameters obtained for the 12 months controls and rats exposed to Jeffrey chrysotile were used to calculate the endothelial cell number for their respective groups at this time period. The pooled endothelial mean nuclear diameter was used to calculate endothelial cell number for controls and dusted rats at all other time periods.

TABLE 8

HARMONIC MEAN TISSUE THICKNESS OF THE AIR-BLOOD BARRIER
DURING AND FOLLOWING ASBESTOS INHALATION

Air to plasma

MALES

3 months	.379±.030	.397±.016	.367±.004	.478±.020*#
12 months	.336±.009	.440±.016*	.485±.017*§	.668±.044*#§
12 months + 12 mo air	.368±.013	.499±.027*	.580±.023*§	.707±.044*#

FEMALES

3 months	.339±.006	.408±.005*	.402±.017*	.402±.016*
12 months	.318±.005	.442±.014*	.474±.008*§	.629±.030*#§
12 months + 12 mo air	.373±.019	.448±.023*	.537±.025*§	.599±.018*

* $p < 0.05$ when comparing to the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA.

$p < 0.05$ when comparing groups exposed to Jeffrey chrysotile with all other exposure groups.

§ $p < 0.05$ when comparing one treatment group to its corresponding treatment group of the preceeding time point.

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TABLE 9

SILICA CONTENT IN THE LUNGS OF RATS EXPOSED TO CHRYSOTILE ASBESTOS

GROUP	N	UGM SILICA/BOTH LUNGS
Controls		
3 + 12 months	17	156 ± 32
Coalinga chrysotile		
3 months	6	610 ± 54*
12 months	8	574 ± 48*
UICC B chrysotile		
3 months	6	569 ± 79*
12 months	8	701 ± 60*
Jeffrey chrysotile		
3 months	8	741 ± 46*
12 months	8	1127 ± 66*#

All data are mean ± SEM.

* p < 0.05 when compared to the controls.

p < 0.05 when compared to all other exposure groups.

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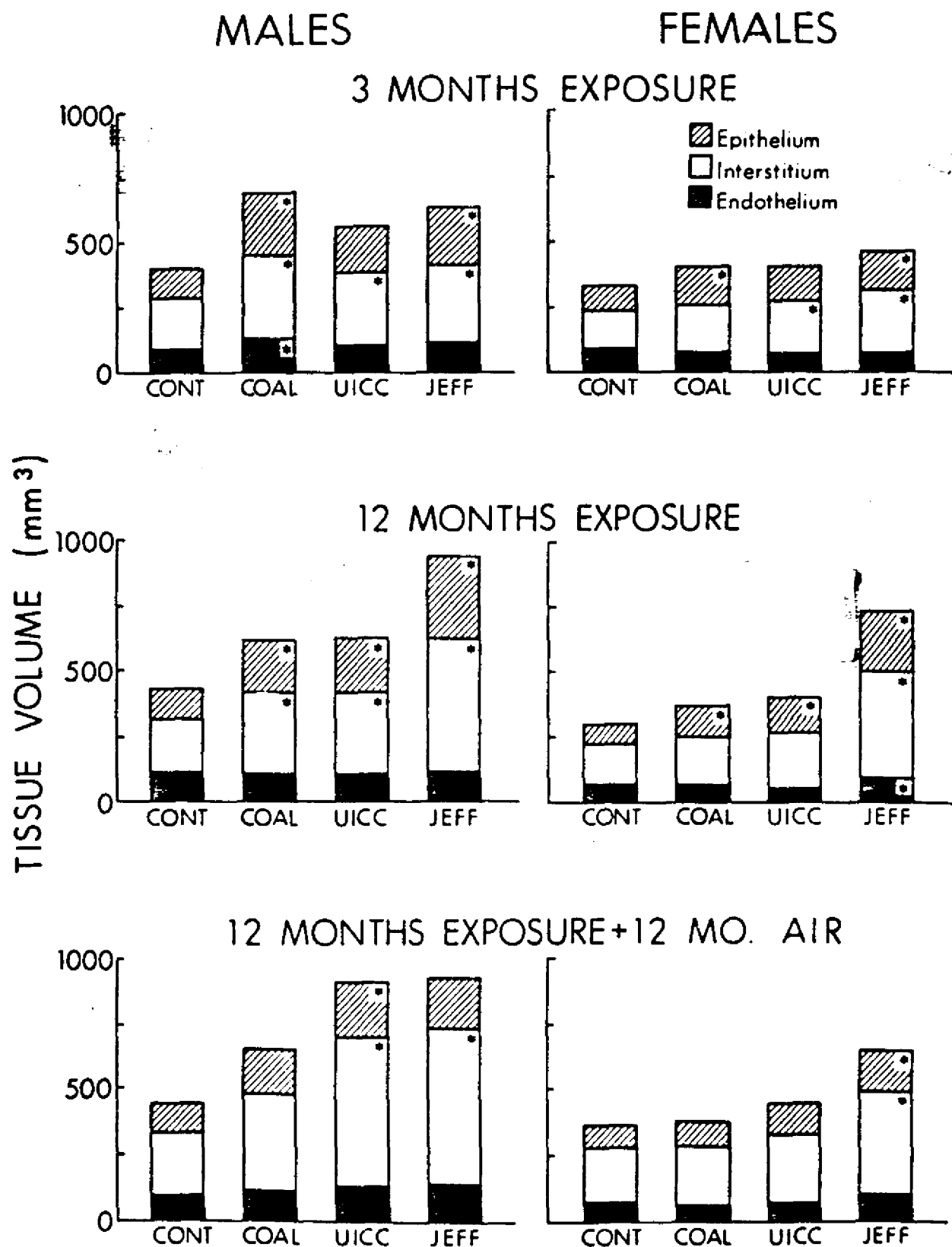


Figure 1: Changes in the volume of parenchymal lung tissue following inhalation of three types of chrysotile asbestos (CONT - controls, COAL = Coalinga, UICC = UICC B, JEFF = Jeffrey). Each bar represents the mean of 4 animals and is subdivided into the epithelial, interstitial and endothelial compartments. *p < 0.05 for comparison to the age-matched controls.

A17847

UCC 007721

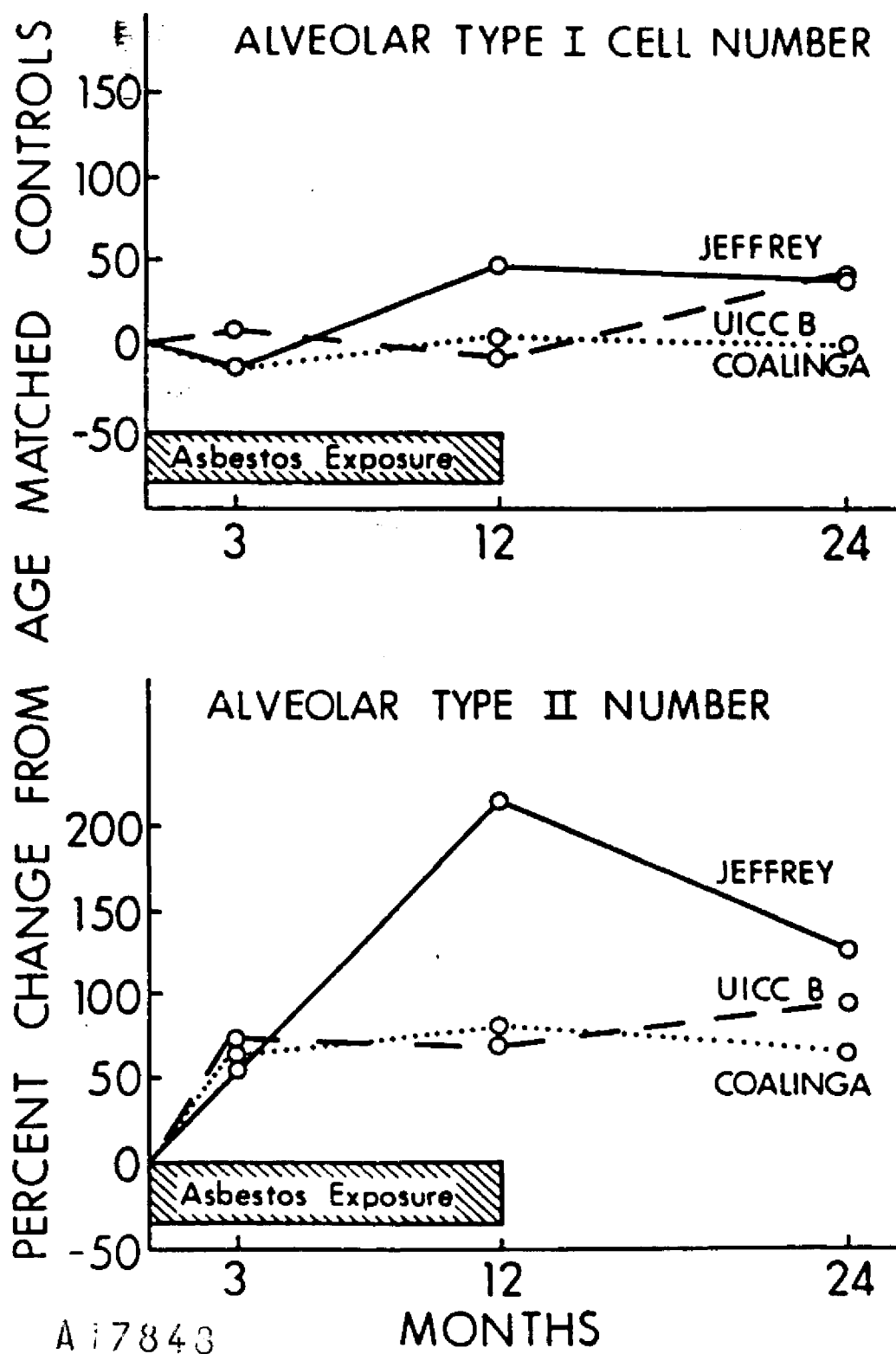
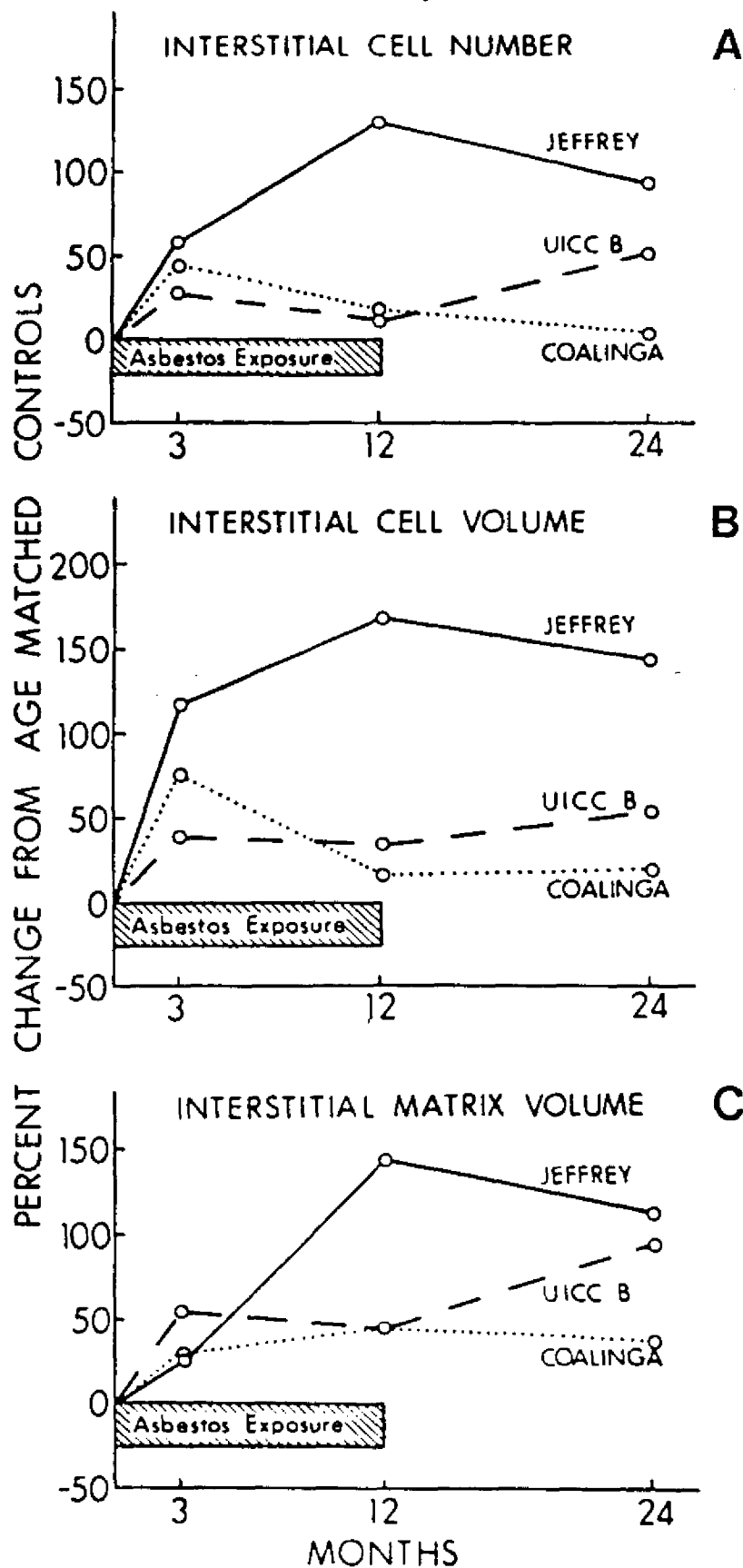


Figure 2: Percent change compared to age-matched controls in the number of alveolar type I (A) and alveolar type II (B) cells in the lungs of rats exposed to Coalinga, UICC B and Jeffrey chrysotile. Each point represents the pooled mean of 4 males and 4 females.



A 17843

Figure 3: Percent change compared to age-matched controls in the number (A) and volume (B) of interstitial cells and volume of interstitial matrix (C) in the lungs of rats exposed to Coalinga, UICC B and Jeffrey chrysotile. Each point represents the pooled data of 4 males and 4 females.

UCC 007723

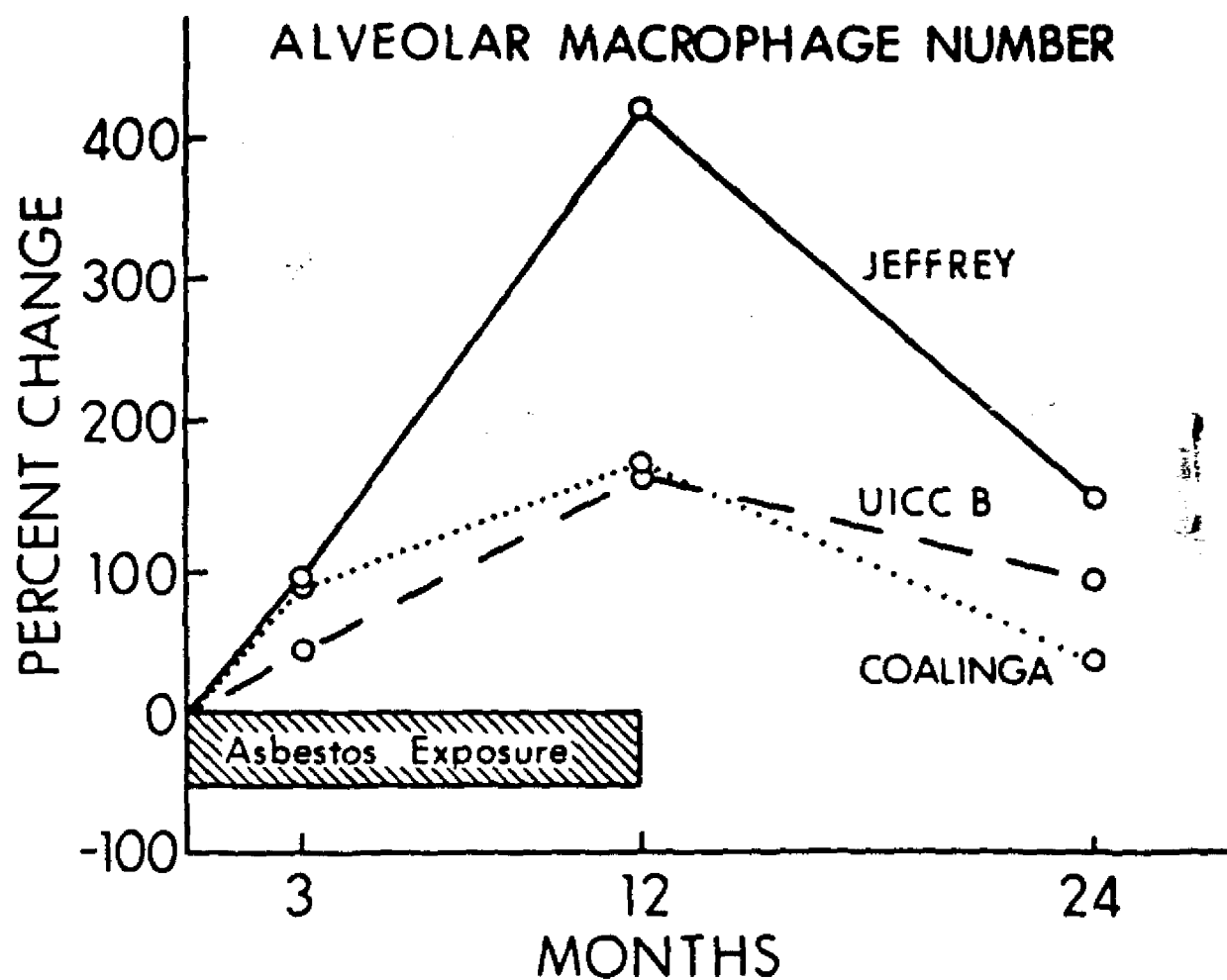


Figure 4: Percent change compared to age-matched controls in the number of alveolar macrophages in the lungs of rats exposed to Coalinga, UICC B and Jeffrey chrysotile. Each point represents the pooled mean of 4 males and 4 females.

A17850

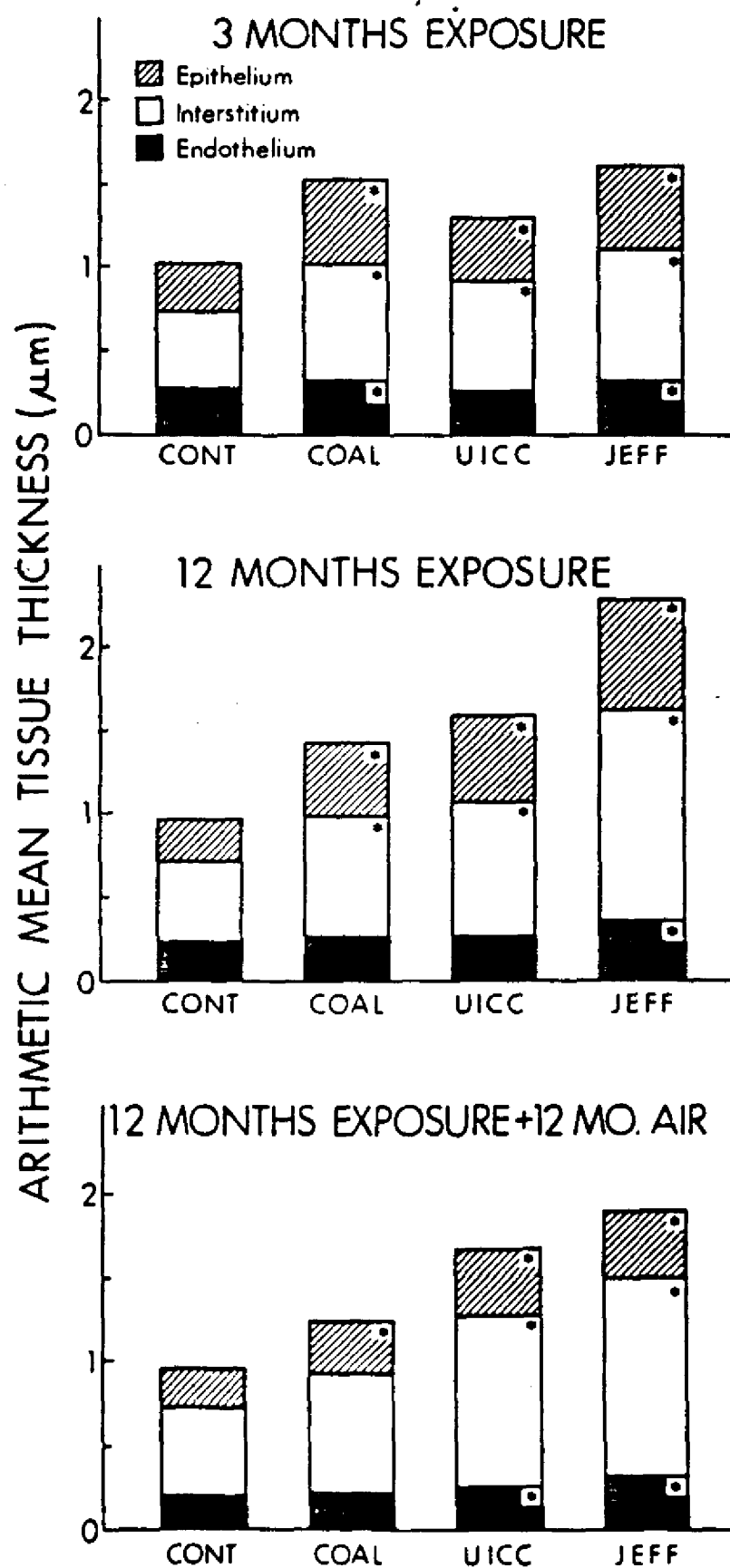


Figure 5: Arithmetic mean thickness of alveolar tissue in control and chrysotile dusted rat lungs. (CONT = control, COAL = Coalinga, UICC = UICC B, JEFF = Jeffrey). Each bar represents the pooled mean of 4 male and 4 female rats. * $p < 0.05$ for comparison to the age-matched controls.

A17851