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Critical controlled inhalation study of pathogenesis of neodymium oxide in mice and guinea pigs

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CRITICAL CONTROLLED INHALATION STUDY OF PATHOGENESIS OF NEODYMIUM OXIDE IN MICE AND GUINEA PIGS

by

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INTRODUCTION

Those elements with atomic numbers 57 through 71 are called the rare earths. They are earths in name only, this terminology arising from the fact that they were first identified as a mixture of oxides that resembled the alkaline earths (Levy, 1924; Yost, 1947; Moeller, 1956; Stevenson and Nervik, 1961). They are rare only in name because of problems of availability and purity which for many years prevented their separation from a common ore. During recent years remarkable strides have been made in processes of chemical separation (Tompkins et al., 1947; Spedding et al., 1947; Marsh, 1952; Wheelwright and Spedding, 1955; Weaver, 1956).

Since the rare earths comprise about one-sixth of all the natural elements, their sudden availability in pure form has been of great interest to the scientific and industrial community. They have found uses in nuclear engineering, in electrical and communication components such as the LASER, in optical systems both in polishing and as coatings, and in metallurgy in alloys and in processing methods. Other major uses include ceramics, carbon arc lighting, incandescent gas mantles, lighter flints, textile water proofing and paint dryers. Secondary outlets greatly extend this list, and an enlarged segment of workers is exposed to various chemical forms of rare earths.

In addition to the industrial uses referred to above, much emphasis has been placed on the study of rare earths in biological systems since they account for a large fraction of fission-product radioactivity. This activity calculated from data reported by Hunter and Ballou (1951) represents about 37, 51, 38, 64, 71, and 41 per cent of the total at

10, 30, 90, 365, 1095, and 3650 days, respectively, after nuclear detonation.

Even though the present accent on the rare earths is primarily an outgrowth of nuclear technology, these elements have been investigated from a biological and pharmacological aspect by several workers over a long period of time. An extensive review by Steidle (1935) summarized the early work and more recently there has been a surge of work with attending biochemical studies. None of these published reports offer information on the effects of stable neodymium oxide when taken in by the pulmonary system. In fact this route of exposure, which would seem to be one of the most common, has been largely avoided by workers because of the problems inherent in inhalation toxicity study methods.

This research evaluates the effects of a dust aerosol of stable neodymium oxide of a known particle size range and concentration on the lungs, other major organs, blood and body weight of mice and guinea pigs when administered over various periods of time up to 120 days.

This information combined with that from studies in other species of animals and under other conditions, should assist in the complete understanding of these elements as possible industrial or public health hazards.

LITERATURE EVALUATION

The literature evaluation will not be confined to neodymium but will cover work done on those elements (atomic numbers 57 through 71) which are termed the lanthanons (Marsh, 1947; Vickery, 1953) and yttrium which also is in Group III A and which has many chemical properties similar to the lanthanide series of rare earths (Moeller, 1956). These elements are similar in their patterns of distribution (Hamilton, 1947), industrial uses (Vickery, 1956), and occurrence in mixed fission products (Hunter and Ballou, 1951; Strominger et al., 1958). For these reasons much of what is known about one can be related, in general, to the others.

It is of interest to note initially that the rare earths and yttrium do not have a known metabolic role in living organisms (Krumholz et al., 1957). In fact, even though these elements are widely present in the earth's crust they are not found in plants. Their absence is, therefore, not from a lack of availability but indicates that plant systems actively discriminate against their absorption by root systems. Their absence in plant tissues generally precludes their presence in animal tissues. For this reason it becomes even more important to determine what effects these elements have when they are made available to the animal or when they are introduced into metabolic pathways.

Work with microorganisms has resulted in several interesting and seemingly fundamental observations. Johnson and Kyker (1961) found that 24 widely representative examples of microorganisms (bacteria, yeasts, and molds) actively took cerium-144 from a mixed fission product contaminated medium in preference to the other mixed fission products. This work

confirmed reports by Richards and Troutman (1940) with lanthanum and by Bowen and Rubinson (1951) who also demonstrated that yeast takes up lanthanum selectively. This latter work was particularly interesting in that the observation was made that the uptake was by yeast which was in turn taken up by larval Drosophila. This demonstrated that the rare earth elements can be made available through existing food chains.

Intensive analytical efforts on human tissues (Tipton et al., 1956, 1957a, b, c; Griffith et al., 1954; and Koch et al., 1956) have not demonstrated lanthanons by emission spectrographic analytical methods. A recent report (Brooksbank and Leddicotte, 1953), has demonstrated by actuation analysis small amounts (average 10.3 μg/g of bone) of rare earths in human bone.

A report by Steidle (1935) observed that even though these elements were not found as consistent components in any living system and even though no functional role could be speculated upon, their chemical properties enabled one to anticipate many possible biochemical interactions. This report emphasized that these interactions could involve nucleoproteins, plasma proteins, phospholipids, enzymes, amino acids, intermediate metabolites, and inorganic anions.

The above interactions are of interest as they affect the actual distribution and excretion of the rare earths. Distribution and excretion studies have involved the use of their radioisotopes in most cases. This has occurred for two obvious reasons. The first is the increased ease and accuracy with which distribution and excretion studies can be carried out with tracer methods, and the second is the current interest in the radioactive lanthanons produced by fission reactions. This

method superimposes radiation toxicity on the chemical toxicity of the elements and should be balanced with stable studies.

Since the rare earth cations are not normal constituents of living systems, their action in the body must be interpreted through knowledge of their chemical properties. It is also necessary to understand that even though these elements are chemically similar there are enough differences, when they are introduced into living systems, that few all inclusive statements may be made for the group. For example Graca et al. (1957 and 1962) made the observation that different stability constants for the rare earths with ethylene diamine tetracetric acid (edetate) and with citrate substantially affected the toxicity of these elements when given intravenously or intraperitoneally. Moeller (1953) reported in a compendium covering the properties of the rare earths that changes occur or properties are graded with increasing atomic number. The atomic radii decrease progressively with increasing atomic number, and with this decrease in radius are seen decreasing basicity, ionic characteristics, thermal stability, and solubility; and increasing acidity, covalent characteristics, and stability of complex ions.

It is of importance chemically that the rare earth cations tend to hydrolyze at the pH of most living systems (Moeller and Kremers, 1944). These hydroxides are relatively insoluble and they precipitate. For this reason Graca et al. (1962) reported that it was not possible to accurately determine the toxicity of the rare earth chlorides when injected intraperitoneally into the mouse and guinea pig. Under these conditions the rare earth was hydrolyzed and precipitated and produced a foreign body peritonitis in the experimental animals which obscured the chemical

toxicity of the lanthanon administered.

The graded, increasing stability of complex ions affects the toxicity of edetate and citrate complexes with rare earths when they are injected intraperitoneally. To demonstrate this fact Graca et al. (1962) found that the elements terbium through lutecium and including yttrium were tolerated by mice at dose levels of 500 mg/Kg of body weight with no apparent ill effects while the LD_{50} for lanthanum in the same experiment was 37.20 mg/Kg with a standard error of $\pm$ 2.17.

The effect of chelation on rare earth ions has been the subject of study not only as a method of administering the lanthanons for toxicity studies but chiefly as a method of detoxication or the removal of the radioactive elements from the mammalian body. The trivalent rare earth cations show a strong tendency to form complexes with several chelating agents. The internal behavior of these chelates varies with the properties of the complex. Some chelates are readily soluble at the pH of tissue fluids and can be easily transported and excreted. Reviews by Foreman (1954) and Schubert (1955) deal with the medical aspects of chelation emphasizing their use in decontamination or removal of radio elements from the mammalian body.

In order to anticipate or interpret effects in the body, the rare earth cations have been studied in relation to their biochemical interaction with specific materials available in the body. Two inorganic anions regularly present, hydroxyl ions and orthophosphate ions, each yield quite insoluble products unless overbalanced by the formation of a soluble complex with some other constituent of body fluids. In a test of 50 or more biochemical compounds for precipitation upon adding

0.1 M thorium, lanthanum, or uranium solutions, Neuberg and Grauer (1953) showed that lanthanum gave a precipitate with almost every compound including Krebs-cycle acids, fatty acids, fruit acids, bile acids, sugar phosphate esters, and other metabolic compounds. Even though their study utilized concentrations of reactants higher than those one would expect to find in vivo, even after large doses of a rare earth, the findings have a far reaching implication as far as possible disturbed metabolic equilibria are concerned.

The structural nature of chelating agents, such as edetate which effectively bind the rare earth cations, would indicate that amino acids would also interact with these ions. This binding does take place and could be of importance since the amino acids exist in a dynamic pool in the body in which free amino acids are ever present in small quantities in the body fluids. That this binding takes place has been proven by several workers. Kawin (1953) observed what he interpreted to be a cationic complex of radioyttrium in paper chromatograms of urine from rats given the element intravenously. In an experiment using 0.001 M solutions of amino acids as eluants for lutecium-177 on a resin column Kyker and Stewart (1955) observed elution by arginine and histidine. At higher concentrations aspartic acid and glycine also removed the lutecium. Other amino acids did not elute the tracer. These findings supported work by Vickery (1950) who demonstrated complexing with amino acids with the lighter rare earths. Loos (1956) also reported that in paper electrophoresis lutecium-177 moved with glycine. Evidence of binding with amino acids indicated that the effect of lanthanons on simple and conjugated proteins could be of importance. Work done with these two classifications

of proteins demonstrated that binding does take place but does not point to a specific metal protein combination (Kyker et al., 1955, 1957). Rare earth chlorides applied topically to the eyes of rabbits (Grant and Kern, 1956) produced an irreversible altering of the protein resulting in corneal damage. Of interest, also, was the ability of lanthanum, praseodymium, neodymium, samarium, and yttrium to give insoluble fibrous precipitates with desoxyribonucleic acid (DNA). In a study by Stern and Steinberg (1953) it was shown that most of the sodium of the original sodium DNA was exchanged by the rare earth. This ability of lanthanum to react with nucleic acid to form almost insoluble compounds has been used for many years in the study of chromosomes and chromatin fibrils (Caspersson et al., 1935; Opie and Lavin, 1946). The implications of this interaction in vivo are far reaching. If the lanthanon ion is made available to the nucleic acid it could result in chromosomal damage and possible alteration of the genetic make-up of the cells. In light of current thinking this could give the lanthanons a carcinogenic capability.

Kerner and Anderson (1955) by culturing Endamoeba histolytica on a synthetic medium and using ultraviolet, radioactive, and staining techniques found that yttrium and cerium ions combined quantitatively with the nuclear constituents of Endamoeba histolytica forming complexes. This evidence indicates that rare earth ions can enter the nucleus of the cell and exhibit their effect.

Interaction with proteins would suggest that the lanthanons would react directly with critical factors in enzyme systems. The rare earths have been shown to affect these systems both by activation and by inhibition. However, observations have been largely without explanation of mechanisms

and have been more or less empirical. Horecker et al. (1939) studied the stimulatory effect of several metallic ions, including lanthanum and neodymium, on the activity of the succinic dehydrogenase-cytochrome oxidase system extracted from beef heart muscle. The two lanthanons accelerated the rate of oxygen uptake, but they could not be found in extracts of the tissue. It is possible that they acted in an indirect way by making available intermediate compounds. Stern (1956) described an accelerated oxygen consumption of Ehrlich ascites tumor cells in vitro in the presence of lanthanum. These tumor cells exhibited a considerable oxygen consumption in a glucose-free medium, while in the presence of glucose this endogenous respiration was partially inhibited. It was found that lanthanum chloride increased the respiratory rate of these cells in the presence, but not in the absence, of glucose. It was suggested that lanthanum inhibited the utilization of glucose by these cells thus obliging them to carry on their endogenous respiration.

Olivard (1960) reported that yttrium-90 forms complexes with phosphate containing anions with appreciably smaller dissociation constants than those observed with strontium-90. The small constants for yttrium-90 suggest that it may be a strong competing ion in biochemical reactions involving phosphate containing anions. This finding suggests that binding of an essential phosphatic part of an enzyme system by a rare earth must be considered as a limiting factor as well as the effect on the protein moeity. In this way enzymatic reactions could be inhibited.

Clayton (1959) found in connection with tobacco root nucleotide pyrophosphatase that the chlorides of praseodymium, lanthanum, gadolinium, neodymium, and yttrium were potent inhibitors of ATP- and DPN-pyrophosphatase

activities. Other dehydrogenase and deoxyribonuclease activities were most marked whereas invertase and polyphenol oxidase appeared to be quite insensitive to relatively high levels of rare earth chlorides. Of the dehydrogenases studied by Clayton, yeast alcohol dehydrogenase was the least sensitive to rare earth inhibition. Moreover, the inhibition of this enzyme could be reversed by the addition of .01 M edetate.

Kyker and Cress (1957) found that the intravenous administration of various rare earths at dosages of 3-4 mg/Kg as the chloride caused the oxygen consumption of liver slices to be depressed. Minimum oxygen consumption was reached on the second or third day and was followed by gradual recovery. The pattern of serum proteins was also distinctly altered with the alpha-1 and gamma globulins increasing and albumin decreasing. In this study approximately 70 per cent of the cerium given localized within a few hours in the liver and remained at that site even after other parameters had returned to normal.

Work by Bamann (1954) showed that the salts of rare earths could under physiological conditions split a wide variety of phosphate esters (glycerophosphate, glucose-1- and 6-phosphate, adenosine triphosphate, nucleotides, and nucleic acids) and for this reason they could be used as enzyme models. He also reported that binding the salt to a hydroxy-carboxylic acid such as citrate destroyed this catalytic activity. As an explanation, it was suggested that colloidal metal hydroxides were formed which reacted with the substrates. The resulting salts were non-dissociated and unstable under physiological conditions. Therefore, they split spontaneously to form phosphate, glycerol, and the original metal hydroxide. Complexing the ions with citrate interfered with the chemical

reactions by blocking them before the formation of colloidal hydroxides.

The validity of these observations was strengthened by Trapmann (1959a, b) who reviewed the literature and extended the concept of rare earth salts as enzyme models. Since all of their physical constants are not known, a stepwise appraisal of the effects of rare earths on the many equilibria that could be affected in the body cannot be made at this time.

The literature reviewed thus far amply demonstrates that the rare earths can and do produce effects in living systems, and their interactions with many normal components of the body indicate that their potential for producing pathological changes is good. For any substance to produce these changes it must find its way into the body. The work of Kerner and Anderson (1955), mentioned earlier, indicated that the rare earths can enter the cytoplasm and the nucleus of individual bacterial cells. This is a simple process when compared with the more complex systems needed in the mammalian body for absorption of some elements. Almost all of the work reported to date on uptake from normal routes (i.e., oral and inhalation) has been done with the radioactive isotopes of the rare earths. In a like manner most of the distribution and excretion studies of these elements have been carried out with active isotopes. Most of this latter work has been done in the medical field in an effort to find elements that can be concentrated in target organs for selective radiation therapy (Brucer, 1952). Data presented in studies of this type must be interpreted in light of the fact that stable and active isotopes may be absorbed, metabolized, distributed and excreted in different manners. Lewin et al. (1954) have shown, for

instance, that even the absorption rate of intraperitoneally administered yttrium-90 is reduced by the presence of stable yttrium.

In general, work with radioactive isotopes of rare earths has shown that they are very poorly absorbed, if at all, from the gastrointestinal tract. Some work with stable rare earth compounds also shows that absorption is poor. Cochran et al. (1950) presented data on the acute toxicity of six lanthanum compounds given orally. These data show that lanthanum salts are of generally low toxicity when administered orally to rats. They speculated that this was caused by poor absorption. Hamilton (1943) reported that the total retention of the chloride of lanthanum-140 was only 0.3 per cent four days after oral administration in rats. In later work Hamilton (1947) reported absorption of less than 0.05 per cent of orally administered yttrium or cerium.

In a comparison of yttrium-91 with strontium-90 absorption by the fish Tilajua mossambica Boroughs et al. (1956) showed that radioyttrium was very rapidly excreted. After two days the fish retained only about 2 per cent of the ingested dose. This was much less than the amount of strontium-90 retained. Their data indicated that most of the yttrium was retained in the viscera and that the 2 per cent figure is probably high due to the fact that fish swallow water to maintain osmotic equilibrium. The water in this case would contain some of the excreted isotope and this process would constitute a recycling of the yttrium-91. These workers concluded that yttrium-91 may have little direct effect on man compared with the possible effects of strontium-90, but the retention of this and other short-lived fission products in marine organisms having a brief life span may possibly affect the biota, and thus affect man indirectly.

The other major route by which exposure to the rare earths could occur by accidental contamination is the pulmonary system. Again work has been primarily with radioactive lanthanons. Schubert (1951) stated that following inhalation exposure to radioisotopes this material appeared in the lungs and the upper respiratory tract. The cilia of the naso-pharynx, the lower part of the vestibule of the larynx, the trachea, and expecially the large bronchioles propelled material toward the mouth where it was swallowed, and then absorbed or passed with feces. In addition to ciliary action, peristaltic motion in the bronchioles, the cough reflex, and nasal hair also removed material. In addition to these, phagocytic cells were said to transport particles to the ciliated surfaces of the respiratory bronchioles where they were subsequently removed by ciliary action. Material not removed mechanically could have left the lung in two ways. The foreign particles might have been engulfed by phagocytes and carried into the lymphatic system of the lung by migration through the alveolar lining, or they might have been dissolved and absorbed into the blood stream. Movement and distribution within the lung were largely functions of particle size. The chemical properties of the lanthanons indicate that within the respiratory passages they would react as colloidal or particulate material.

Cohn and his co-workers (1956) carried out a study of the uptake, distribution and retention of three day old fission products in mice exposed to an aerosol with sea water as the carrier which included mixed radiolanthanons. This work does not treat these elements separately and gives no information pertinent only to the rare earth group of elements.

In an attempt to estimate the acute toxicity of insoluble, radioactive material in the lungs Seibert and Abrams (1946) exposed rats to smokes containing cerium-144. Rats weighing 200 grams received doses of 3.2, 14, 39, 50, and 200 μc/rat. This experiment demonstrated slow absorption from the lung with primary deposition of the absorbed material in the liver and the skeleton. Because of the problems associated with the determination of the administered dose and its quality, in terms of particle size and fraction ingested, the estimates of rate of mobilization from the lungs are somewhat uncertain. The biological half time in the lung could not have exceeded 50 days and was possibly less. Hamilton (1943) found the biological half time of cerium in the lungs (introduced directly in solution as the chloride) to be only 14 days. The lesions noted in the experiments by Seibert and Abrams were clouded by an exacerbation of an endemic pneumonia that was prevalent in their animal colony at that time. Squamous metaplasia of the bronchial mucosa was observed more extensively in the experimental animals than in the controls, and many atypical epithelial cell types were noted. Norris et al. (1956) in reporting on the completed work of Seibert and Abrams stated that many of the animals died with malignant epithelial tumors of the lung, whereas no tumors of this type were observed in the controls. There was no effort to establish whether the tumor production was a function only of radiation damage or whether it was influenced by the chemical toxicity of the cerium as well. Vorwald et al. (1963) exposed albino rats and guinea pigs to concentrations of 72 mg/ft^3 of stable yttrium oxide. Groups of rats were given single exposures for 5, 15, and 30 minutes; multiple exposures (6, 12, 18) for 5 minute periods and 18 exposures of 30

minutes each. A series of guinea pigs were exposed to the same aerosol concentration of yttrium oxide for a single 30 minute period and subsequently maintained for periodic sacrifice for chemical and histological analysis. An additional experiment was performed in which 30 albino rats were exposed to yttrium oxide aerosol for 30 minute periods at monthly intervals. Tissues examined from this experiment have shown increasing amounts of particulate matter with persistence of a nodular reticulo-endothelial hyperplasia in the tracheo-bronchial lymph nodes. Lung changes were mild and consisted of some early septal fibrosis and occasional epithelial hyperplasia in guinea pigs. This preliminary report by Vorwold and his co-workers indicates that clearance of stable yttrium is similar to that for the isotope yttrium-91. Exposure in this experiment was a nose-only exposure with anesthetized rats. There was no information supplied concerning the particle size of the yttrium used.

Other routes of exposure to rare earths such as interstitial or intracavitary and intravenous have been much more thoroughly investigated than the oral and pulmonary routes. These studies have been carried out in most cases with radioisotopes and have been aimed at selective localization of the active elements for medical purposes. The portion of this work that is of interest in this dissertation is that which deals with distribution studies. The permeability of tissue barriers, the solubility or physical state of the rare earth used and the mechanisms available for transport of the element or compound all affect the dose which actually finds its way into the system. If the rare earths are systemic, they follow a general pattern of distribution. Durbin and co-workers (1956 a, b, 1957) reported on distribution and excretion studies

when different routes of administration were used (intramuscular and intravenous) and with citrate chelated and non-chelated cerium-144, europium-152, terbium-160, and thulium-170. Distribution was similar for the absorbed fractions of the intramuscular dose and the intravenous dose with the liver and skeleton accounting for 70 per cent of the fraction at 24 hours. The amount in liver decreased sharply with increasing atomic number of the four elements (from 54 to 4 per cent) and, correspondingly, the values for skeleton increased (from 29 to 65 per cent). Excretion also increased with atomic number.

Toxicity evaluations have been carried out on the rare earths with only limited explanation of mechanisms at the level of the cellular enzyme system. Graca et al. (1957, 1962), with stable rare earths given intraperitoneally to mice and guinea pigs, demonstrated that these elements administered as the chloride, citrate, and edetate complexes produced graded toxic reactions in doses from 25 mg/Kg to 500 mg/Kg of body weight. Rare earth chloride toxicity was difficult to evaluate because of hydrolysis and precipitation at the site of injection with subsequent development of peritonitis. The citrate and edetate complexes were absorbed from the injection site and showed a general pattern of decreasing toxicity from the lower to the higher lanthanons. In these experiments liver pathology was present as a constant finding in animals which succumbed.

The effect of rare earths on hepatic tissue has been studied rather extensively by Kyker and his co-workers (1957) and Snyder, Cress, and Kyker (1959, 1960). These changes are expressed as an acute fatty infiltration of liver in rats. Total liver lipids reached a maximum of three

to four times normal values within three days and returned to normal by seven days. Graca et al. (1962) also reported that guinea pigs injected intraperitoneally with rare earths, which died within the first few days after injection, had hepatic lesions. Remarkable regeneration had occurred in livers of animals that survived and later were sacrificed at 168 hours. Efforts to clarify the mechanism of this acute disorder seem to rest on two postulates; one, that utilization of liver lipids is inhibited and, another, that mobilization from lipid depots is responsible. This question does not seem to have been resolved.

Rare earths also have an anticoagulant ability that is not entirely understood chemically. The effect of rare earth salts, especially neodymium, on the blood, has been known for a long time. Guidi (1930) and Steidle (1935) observed that neodymium delayed the coagulation of blood. Dyckeroff and Grunewald (1943) studied the anticoagulant effect of neodymium, praseodymium, erbium, and samarium in vitro. It was suggested that the anticoagulant effect resulted from antiprothrombin activity.

Vinck and Sucker (1950) succeeded in developing the compound neodymium nicotinate and, more recently, a neodymium salt of 3-sulfo-isonicotinic acid which had high solubility and almost no toxic effect in physiological concentrations. They also concluded that neodymium had interfered with the action of prothrombin. Hunter and Walker (1960 a, b) injected neodymium 3-sulfo-isonicotinate intravenously in men and rabbits and found that the rare earth salt inhibited Factor IX (Christmas factor) and Factor X, while reducing partially the activity of Factor VII. With a higher dose, the clotting time of whole blood was prolonged and the activity of the antihemophilic globulin factor was reduced. Thrombin

activity remained normal. It was suggested that neodymium acted as an antimetabolite of calcium merely by occupying its place in one or more of the protein coagulant factors. Graca et al. [1963] also demonstrated in dogs injected intravenously with rare earths that the changes in clotting time were not entirely attributable to changes in prothrombin even though these levels were affected.

MATERIAL AND METHODS

Experimental Design

In order to accomplish the objectives of evaluating the effects of inhaled neodymium oxide (Nd_2O_3) on the lungs, other major organs, hemogram, and body weight of mice and guinea pigs, it was necessary to complete a preliminary program in which a physical system for accomplishing the experiment could be designed and built and in which a pilot experiment could be conducted to aid in the design of the final experiment.

Inhalation system

Design requirements for the inhalation system were set by the scientific investigator and design itself was the responsibility of an Atomic Energy Commission junior engineer, Mr. Robert Cook.

Detailed information concerning the engineering of the system has been reported by Cook (1961).

The two most important requirements set for the system were safety and reliability. In addition to these all inclusive and general requirements the system had to meet standards in the following additional parameters:

1. control of through-put air
2. control of humidity
3. control of temperature
4. even dispersal of aerosol
5. in-system methods of aerosol sampling
6. satisfactory filtration properties
7. construction with non-reactive or inert materials.

It was decided early in the design phase that the most effective safety factor for people operating the inhalation equipment would be a unit which could operate only under a pressure negative to room atmospheric pressure. A system of this type would insure that any leak resulting in air movement between the room and the test atmosphere in the chambers would be from the room into the contaminated chamber. This major safety factor was insured by the installation of a low vacuum safety switch. The safety switch sensed pressure differential between the inhalation unit and atmosphere by applying the differential pressures to opposing sides of a flexible diaphragm. In the event of a power failure or a loss of vacuum resulting from any cause the entire motor control circuit was shut down. This circuit had a manual reset and, therefore, if power failed and then was reestablished the system would still remain off.

Reliability was viewed as a function of the quality of engineering and construction. Two years of almost continuous operation with no loss of time due to equipment failure have demonstrated that the reliability of this system is excellent.

The design requirement for the system concerning control of air flow was that volumes from 0 to 40 cfm be obtainable and reproducible. Operating conditions in one inhalation unit could not influence those in the other units. Control of this parameter was important for several reasons. Through-put air constituted a method of diluting the test agent introduced into the atmosphere. It therefore became one of the governing variables in concentration control. Air flow is or can be a stress factor superimposed on the experimental animal being exposed to the test material. Air flow also influences the speed with which the dispered

aerosol is moved through the system. This becomes important when the animal being exposed is inhaling particulate aerosols in a plane perpendicular to the overall flow of the aerosol.

The rigid control of air flow necessary was attained by the use of variable speed blowers. These blowers (one for each inhalation unit) were located on the downstream side of the units. Speed control of the blower through a variable speed pulley enabled the operator to adjust the air flow to the nearest cfm. Actual flow was measured in the air supply lines at orifice plates with taps for differential pressure measurement.

The permissible range set for humidity in the chambers was from 30 to 50 per cent. Even though this range was rather large it is in that portion of the humidity scale in which experimental animals are apparently comfortable. Control of this parameter could have been made much more precise, but the cost of this type of control would be almost prohibitive. Based on past experiments with laboratory animals it was believed that humidity fluctuating between 30 and 50 per cent would be comfortable and would not constitute a stress. Changes within this range would be gradual and comparable in the control animals. The above was accomplished by conditioning the air of the inhalation room with a window type cooler. During periods of high outside humidity further conditioning was necessary with an additional modified window type cooler for the inhalation system.

Design requirements concerning temperature were that this parameter be under rigid control at any designated value between 65° and 85° F. Constant temperature was desirable and it was felt that each individual unit should be controlled separately. The system was designed with the

best temperature control at 76° F. This was accomplished by the two coolers mentioned in the above paragraph. The inhalation unit air conditioner was modified to eliminate cycling control. The compressor ran continuously while the unit was in operation. This unit produced constant temperature air leaving the cooling coil. A preheater coil compensated for variation in condition of the room air by adding a greater or smaller amount of heat to the supply air. When full capacity was reached, the hot water supplied to the preheater coil was shut off. A pneumatic proportional controller was used to control the hot water rate to the coil. A hot water booster coil was used downstream from the inhalation unit air conditioner. This booster coil handled the entire volume of air. The air was heated to within a few degrees of the desired inhalation unit temperature. Each branch duct to each inhalation unit had an individual hot water reheat coil which brought the air to the desired final temperature. The temperature of the hot water was maintained in a steam-to-hot water heat exchanger.

These necessary precautions and control measures were taken to assure constant temperature and humidity since experimental animals were to be maintained in the chamber environment for periods up to and exceeding six hours per day for periods of days to months. Since respiratory rate is directly affected by both temperature and humidity and since the dose of aerosol received is in part a function of respiratory rate, it becomes apparent that these two parameters must be controlled to assure a uniform and constant dose of aerosol.

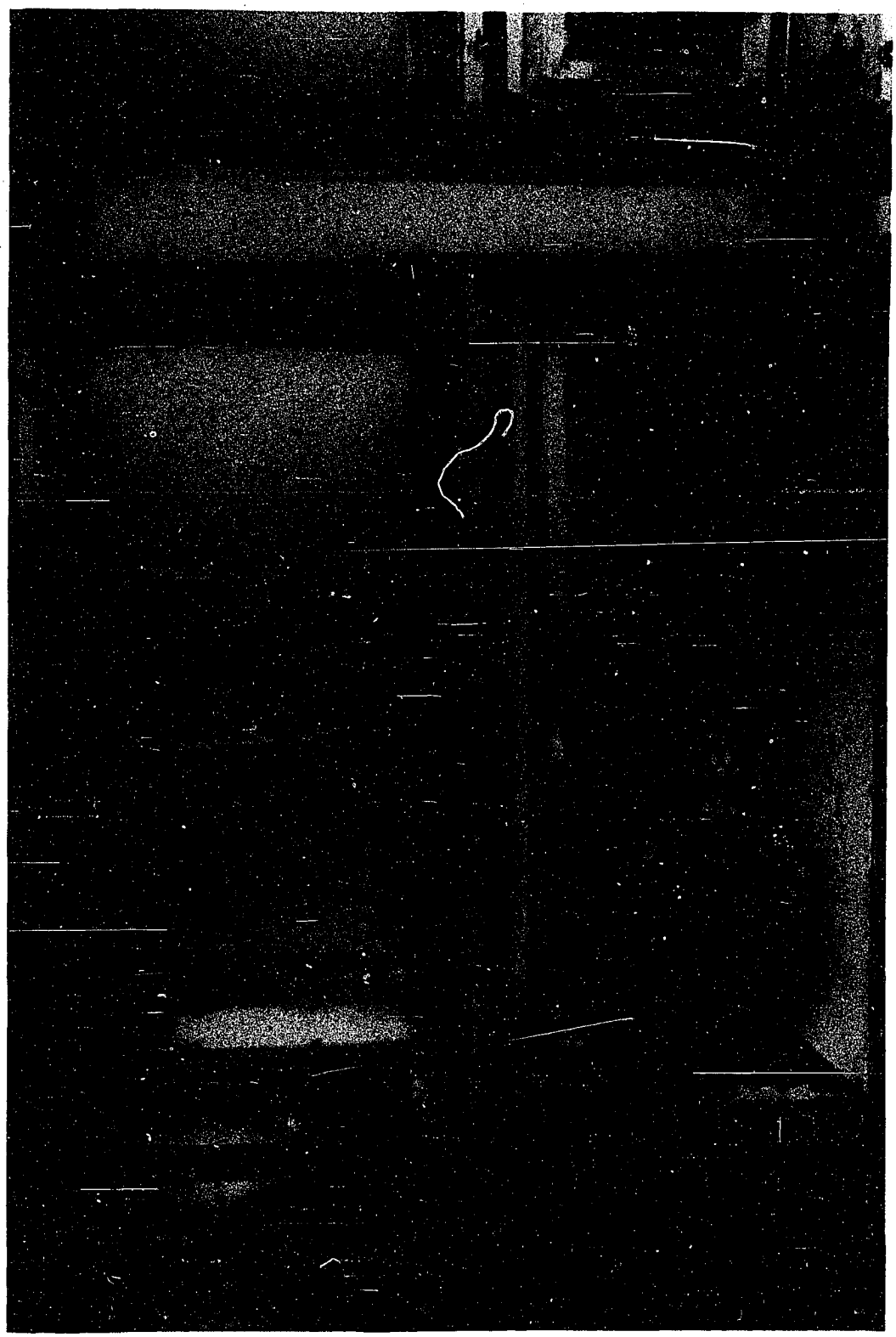
The even dispersal of aerosol was dependent on the design of the inhalation boxes or units. These units were built in a modified cubical

shape. They had rectangular holes in the top and bottom, centrally located, to accommodate the ductwork. The upper front corners of the units were cut to allow the upper front pieces to be placed at an angle of approximately 38° with the vertical. Both the upper and lower front pieces were made of plexiglas to permit observation. The inlet duct opening was equipped with a baffle to distribute the supply air more uniformly throughout the unit. The outlet duct opening was baffled to provide uniform suction conditions.

Determination of the contaminant distribution in this system was the subject of extensive work by Cook (1961). He showed in a sampling traverse of 9 positions in planes normal to the air flow that contaminant distribution of neodymium oxide was uniform. It is believed that, for its size, this unit had as good a standard of distribution as any in existence. The necessity for uniform distribution was obvious since many small laboratory animals were to be exposed in each inhalation unit. This system allowed one to expose animals with assurance that those in different positions in the unit were being exposed to the same concentrations of contaminant. Figure 1 shows one small inhalation unit.

It was believed that sampling of the contaminant concentration within the chamber should be accomplished daily and this should be done without altering the test atmosphere in any way. The prime mover of this system was a vacuum pump. This pump induced vacuum in an air line which was connected to the interior of the inhalation unit. The air flow was from the unit interior into the sampling head, through the flexible tubing, stainless steel sleeve connector, outside copper tubing, sampler solenoid valve and sampling air regulator valve to the vacuum pump.

Figure 1. A typical small inhalation chamber used in this experiment.



The sampling head was a standard stainless steel, Gelman open filter holder[1] for a filter of one inch diameter. This filter holder was loaded each day with a Gelman AM-5 membrane filter.[2] The holder was then capped and suspended from a stainless steel yoke attached to the inner wall of the inhalation unit. During the inhalation period the cover was removed from the filter holder without breaking the air seal by using glove ports in the inhalation unit, and a known amount of air was drawn across the filter by the vacuum pump. Sampling was for a single 15 minute period each day, and the sampling air regulator valve was set to allow a flow of 16.8 ft^3/hour across the membrane filter. Under these conditions the contaminant was removed from .12 $meters^3$ of chamber air. The amount of contaminant on the filter was determined spectrophotometrically using the method of Fritz (1958), and the concentration was expressed as $mg/meter^3$ of air.

Rigid design requirements for filtration were set, and filtration of both supply and exhaust air was accomplished by using Chemical Warfare Service type filters.[3] These units gave efficiency of 99.95 per cent or greater for particle sizes down to 0.3 micron. Prefilters were used upstream from the "absolute" filters to remove large particulate matter and to keep the final filters from loading up. Necessity for this type of filtration is twofold.

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[1] Gelman Instrument Company, Chelsea, Michigan.

[2] Ibid.

[3] Mine safety appliance, Pittsburgh, Pennsylvania.

First, in initial toxicity studies it is important that animals be exposed only to the material being studied. This was necessary since mixed contaminants may act synergistically or even competitively. This system provided as near chemically pure air to the chamber as was possible using Army Chemical Warfare type filters. The contaminant was introduced into this "pure" air.

Second, when unknowns are being evaluated it is necessary to trap the contaminant and release only "clean" air back into the atmosphere. This system stripped out almost all of the test contaminant and the cleaned air was vented outside and released.

All filters were monitored with differential filter pressure gauges. The upstream and downstream filter pressures were admitted to two pressure tight compartments on either side of a flexible diaphragm. As differential pressure changed, the diaphragm flexed. The degree of flexing was recorded through a magnetic system and read as inches of water. This type of monitoring demonstrated when filters were becoming loaded and endangering the efficiency of the system. When filter resistance reached two inches of water, these units were changed. Prefilters were cleaned daily with a vacuum cleaner.

Since air may be contaminated by materials of construction, an initial design consideration was the use of only certain acceptable construction materials. After the final air filtration and prior to inhalation by the animals, stainless steel was the only metal in contact with the air. To provide visibility in the ductwork downstream from the first absolute filter to the chamber, plexiglas was used in one side of the duct. It was also used for the front of the inhalation chamber and in

the duct carrying air from the inhalation unit to the final absolute filter. The inhalation chambers themselves were constructed of five-eighths inch plywood. The interior of this unit was finished with a neutral formica. This provided an interior which was watertight, easily washed, impervious and relatively non-reactive. The units were lighted by exterior fluorescent units sealed from the interior with plexiglas but flush with it. The inhalation units were fitted with a stainless steel support rack at the bottom on which rested the stainless steel cages in which the animals were held. Two circular holes were cut in the lower plexiglas front piece to accommodate glove rings and surgical sleeves. This system presented a closed unit in which the only materials in contact with the internal atmosphere were stainless steel, plexiglas, formica and neoprene sleeves.

The total system of 5 small inhalation boxes and one large unit[4] was operated from a single control panel (Figure 2).

After two years of operation the excellence of engineering concepts and construction is attested to by the fact that these units have never suffered a major malfunction and they have fulfilled the exact purposes for which they were designed.

Pilot Intratracheal Experiment

A pilot intratracheal experiment with rats and guinea pigs was conducted. This was a program designed to give basic information so that exposure levels could be established for the inhalation experiment. It was performed to answer the following questions:

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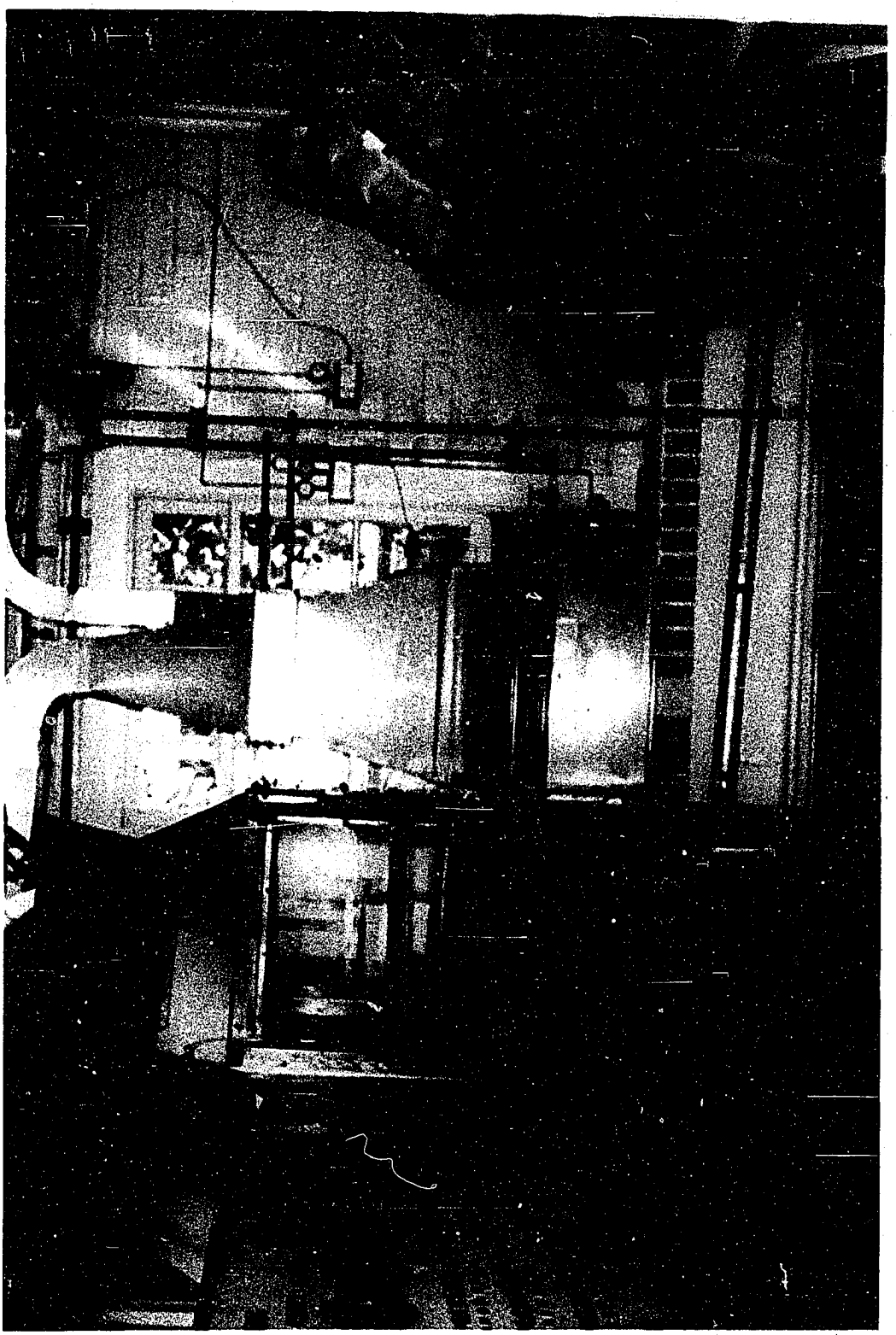
[4]The large inhalation unit was purchased from Wm J. Schmitt, Inc., Rochester 6, New York.

Figure 2. The inhalation facility designed to expose experimental animals to aerosols of the lanthanons. The control pannel on the left serves the single large chamber and the bank of five small chambers. Three of the small chambers are seen on the right.



1. Are stable neodymium oxide and neodymium citrate complex cleared or translocated from the lung?

2. If so, to what other major organs or tissues is neodymium translocated?

3. Is a significant lung burden still present at the end of 120 days following a single exposure?

4. Does chelation with citrate enhance the removal of neodymium from the lungs of rats and guinea pigs?

In this experiment three rats and three guinea pigs were injected intratracheally with Nd_2O_3.[5] The oxide was prepared by grinding in a Pica Blender mill Model 3800[6] for 8 minutes. Stainless steel vials and balls were used. This produced a fine dust from the oxide which later checks by electron microscopy showed to have a preponderance of particles with diameters in the range of 0.1 to 1.0 micron. The oxide was suspended in carboxy methyl cellulose (CMC) as a carrier. This carrier was a polycarboxylic acid similar to the polyglucuronic acid component of normal gastric mucus and was chosen because of its action as a protective colloid, its buffering action and its stability. Three rats and three guinea pigs were also injected intratracheally with the citrate complex of neodymium. Twelve rats and twelve guinea pigs were used as controls. The controls were injected with CMC, with CMC plus sterile talc, and with citrate in the same concentration and same total

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[5]Neodymium oxide was supplied by the Ames Laboratory of the U. S. Atomic Energy Commission, Ames, Iowa.

[6]Pitchford Scientific Instruments Corporation, Pittsburgh, Pennsylvania.

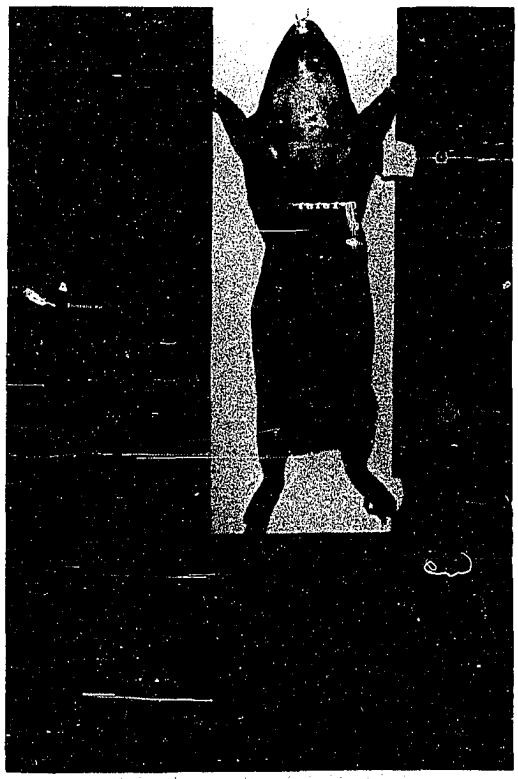
volume as that used in the animals receiving the rare earth. These animals were sustained on a standard laboratory diet.

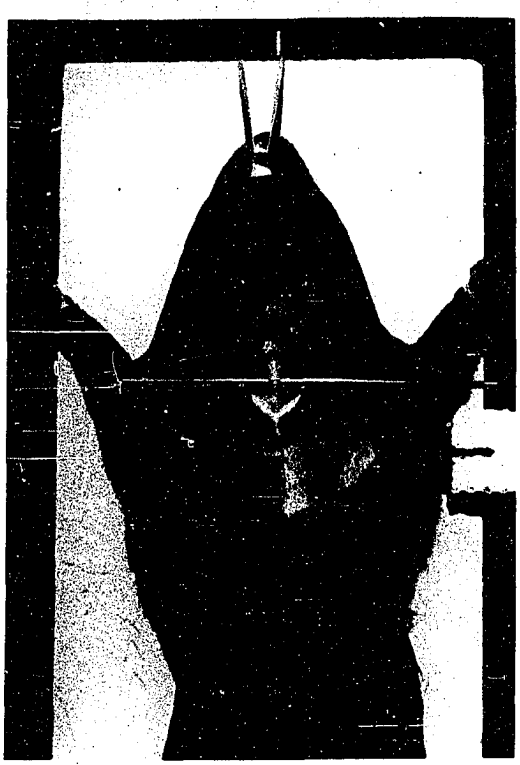
Rats and guinea pigs were given the neodymium oxide at a dose rate of 20 mg/Kg of body weight. This was suspended in 0.3 ml CMC. Animals in the citrate groups were injected with 10 per cent neodymium chloride ($NdCl_3$) chelated with 10 per cent citrate at the ratio of 1:3 and at a dose of 15 mg/Kg of body weight. The discrepancy in dosage between the oxide and the citrate complex groups resulted from the fact that 20 mg/Kg of the citrated rare earth chelate produced high mortality rates even though the controls with the same volume of citrate did not die. Even at 15 mg/Kg of neodymium citrate all three guinea pigs died. The rats survived.

The intratracheal injections were made surgically using aseptic technique. The surgical procedure included anesthesia with pentobarbital sodium at a dose rate of 30 mg/Kg of body weight. This was administered intraperitoneally. When a surgical plane of anesthesia was reached (approximately 20 minutes post injection) the animal was immobilized on a board as shown in Figure 3. The board was inclined at an angle of about 45°. The ventral cervical area was clipped and a midventral cervical incision approximately 1.5 cm long was made. The trachea was isolated by blunt dissection and exposed through the incision (Figure 4). A 16 gauge needle was inserted into the trachea between two cartilage rings. This needle was pushed along the lumen of the trachea for 1.25 cm and a polyethylene tube 3.25 cm in length with an outside diameter of 0.038 inches and attached to a 22 gauge hypodermic needle was inserted into the trachea, past the tracheal bifurcation and into one of the major

Figure 3. An anesthetized guinea pig immobilized on a holding board and prepared for intratracheal injection.

Figure 4. Trachea isolated through ventral midcervical incision.





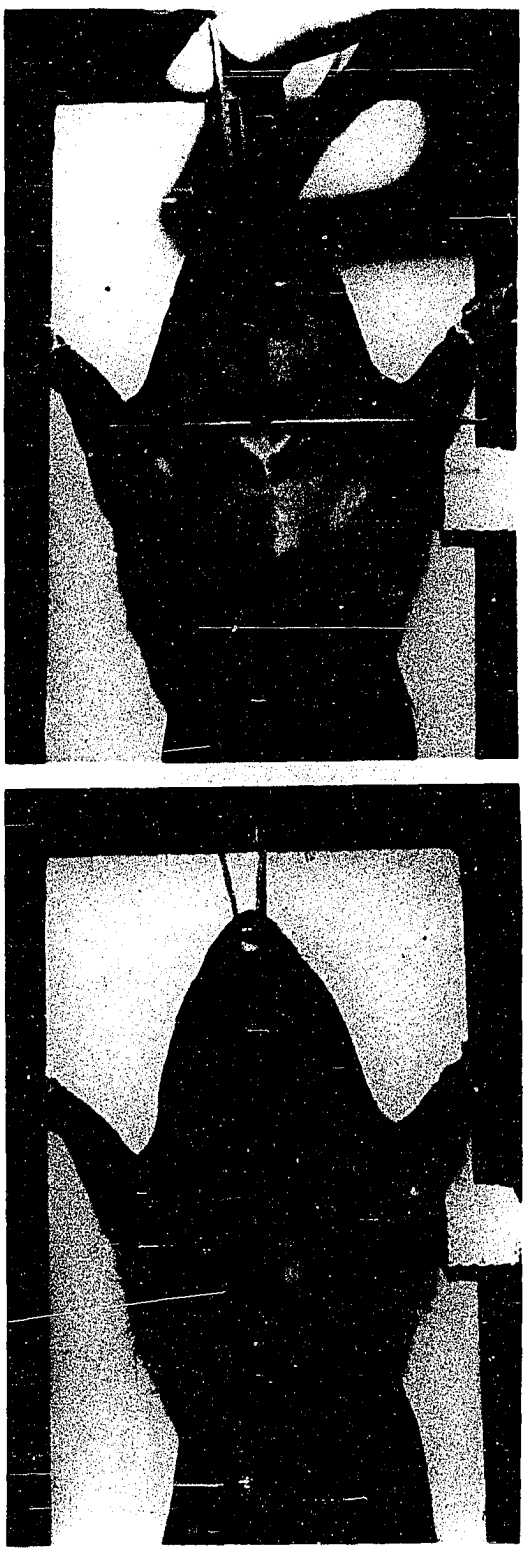
bronchi. At this time the material to be injected was forced into one lung as shown in Figure 5. A 1 ml tuberculin syringe was used to deliver the rare earth. Oxide residue left in the syringe was flushed into the lung with a smaller amount of the carrier used. In all cases the total volume equalled 0.3 ml. After injection the tube was withdrawn and the incision was closed with two or three sutures (Figure 6). Excellent recovery was noted in all animals with no subsequent infection.

One rat and one guinea pig were sacrified from each neodymium oxide group and from each neodymium citrate group at 60, 90, and 120 days. Two rat controls and two guinea pig controls from the citrate group were sacrificed at the same time intervals. All animals were weighed initially and at the time of sacrifice. Whole organs were harvested from all animals for analysis of neodymium. These organs were trimmed of fat and connective tissue and wet weights were determined and recorded. They were frozen in individual test tubes and stored until all the tissues were collected.

At the completion of the sacrifice schedule the tissues were thawed and placed in individual pyrex beakers. They were dried in an oven for 12 hours at 90° C. At the end of this period the temperature was increased to 200° C. for another 18 hour period. This process dehydrated the tissue and burned some of the organic material. It also reduced foaming during the acid digestion which followed. This digestion was accomplished in an exhaust hood with a mixture of 2 parts concentrated sulfuric acid to 1 part concentrated nitric acid. In some samples a low heat was used to initiate the digestive process. After digestion a brownish black solution remained in the beaker. This was transferred to numbered crucibles whose

Figure 5. Intrapulmonary injection of rare earth using a 1 ml syringe.

Figure 6. Intratracheal injection procedure completed. Skin incision sutured.



weight had been determined by firing in a muffle furnace until constant weight was reached; this weight was recorded. Excess acid was driven off by placing the crucibles on an electric hot plate overnight in the hood. This process reduced the solution to a black tarry material. Each crucible was then placed back in the muffle furnace and held at 500° C. for 24 hours. This left a light colored ash on the bottom which only rarely adhered to the sides of the crucible. The crucibles were removed from the furnace, placed in a dessicator and weighed. Ash weights were calculated as differences between the fired crucible weight and the final weight.

Other methods of ashing were tried but the one outlined above gave the most satisfactory results.

The ashed organs were presented to Dr. Velmer H. Fassel of the Ames Laboratory of the United States Atomic Commission. His group determined the neodymium content by mass emission spectrographic methods. The results for the rat organs are shown in Table 1. Results for the guinea pig organs may be noted in Table 2.

Control tissues did not contain any neodymium.

Discussion

Results of this preliminary experiment indicated that:

1. Neodymium oxide and citrate complex were cleared from the lung.
2. It was partially translocated to other organs of the body.
3. Even though it was translocated, there was still a substantial lung burden (greater than 10,000 ppm) remaining after 120 days.
4. Chelation with citrate generally resulted in a higher per cent of neodymium in the other body organs of rats. A significant lung

Table 1. Tissue content of rare earths in rats

Injected with 20 mg/kg of Neodymium Oxide and
15 mg/kg Neodymium Citrate I/T

Expressed as ppm except where noted differently

	Days	Rat	Adrenal	Brain	Femur	Heart	Kidney	Liver	Spleen	Sternum	Testis or Ovary	Lungs
Nd Oxide	60	28	< 100	140	90		< 50	< 50	< 50	140		>1.0%
	90	29		60	90		< 50	< 50	< 190	90		>1.0%
	120	30	< 670	110	180	800	110	< 50	240	70		>1.0%
Nd Citrate	60	25			80	< 500	120	360	640	90	< 400	>1.0%
	90	26		< 80	< 50	< 830	120	390	800	110		>1.0%
	120	27	< 100	< 90	120	< 1400	100	460	610	170	< 490	>1.0%

Table 2. Tissue content of rare earths in guinea pigs

Injected with 20 mg/kg of Neodymium Oxide and
15 mg/kg of Neodymium Citrate I/T

Expressed as ppm except where noted differently

	Days	GP	Adrenal	Brain	Femur	Heart	Kidney	Liver	Spleen	Sternum	Testis or Ovary	Lungs
Nd Oxide	60	28	270	40	110	30	35	25	440	40	45	~2.7%
	90	29		160	140	820	580	110	1540	380	580	>1.0%
	120	30		340	130	170	120	< 50	< 570	160	140	>1.0%
Nd Citrate	60	All guinea pigs injected with Nd Citrate at 20 mg/kg died.										
	90	All guinea pigs injected with Nd Citrate at 15 mg/kg died, and on replication animals at this dose level died.										
	120											

burden still remained.

Gadolinium, cerium, dysprosium, yttrium, and ytterbium were checked at the same time with neodymium. Of these six lanthanons, neodymium and yttrium were the only two in which all guinea pigs injected with the citrate complexes died. However, with all the compounds tested the citrate complex resulted in a higher mortality rate in guinea pigs than the corresponding oxide. When compared to the citrate control animals in which there were no deaths, the above findings would seem to indicate that the citrate complex makes the rare earth available either more rapidly, in a more absorbable form, or that the citrate and the rare earth toxicities act synergistically in the guinea pig. Graca et al. (1962) also found that the toxicity of citrate complex injected intraperitoneally was greater in guinea pigs than in mice. In that report the LD_{50} for neodymium citrate at 168 hours when injected intraperitioneally into guinea pigs was 52 mg/Kg compared to 140 mg/Kg for the mouse. Apparently the guinea pig is less tolerant to neodymium than is the mouse or rat.

In this experiment translocation was evident even though the mechanism was not defined. It is known that some of the material injected into the lung is expelled and swallowed. No effort was made to determine the per cent of the dose that was lost in this manner. There was no way to determine from this work how much, if any, absorption of neodymium from the gastrointestinal tract occurred. Uptake from the gut could contribute to the burden of the organs. However, measurements after oral administration of lanthanon chlorides in other experiments indicated essentially no absorption from the gastrointestinal tract (Hamilton, 1947). This would support the credence that certainly the greater part of the

neodymium found in the other organs or tissues of the body was transported there from the lung by the blood.

Determinations on lung clearance with six rare earth oxides and citrate complexes (Davison, unpublished work) indicate that tissue deposition follows basically the same pattern as with intravenous injections. The heavier rare earths are concentrated more in the hard tissues and the lighter rare earths more in soft tissues.

Inhalation experiment

Using knowledge gained from the intratracheal experiment and using the inhalation equipment designed and tested in the preliminary phase, a more comprehensive program was devised for the study of inhaled neodymium oxide in the mouse and guinea pig.

Final experimental design

Mice This program utilized 144 mice. These animals were exposed to approximately 30 mg of neodymium oxide/meter^3 of air for periods of six hours per day, Monday through Friday. The longest duration of exposure was 120 days. At the end of each 10 day exposure period six mice (three males and three females) were taken off test. At the same time six control mice which had been under the same atmospheric and stress conditions, with the exception that no Nd_2O_3 had been introduced into their chamber system, were taken off test. As the 10 day groups were removed from the chamber environment they were sacrificed. Clinical hematology consisting of white blood cell count, clotting time, hemoglobin, hematocrit and differential white cell count was done antemortem, and gross pathological observations were made at necropsy.

Sternum, heart, lung, liver, adrenal, kidney, testis or ovary, spleen and mediastinal and mesenteric lymph nodes were taken for histopathological evaluation.

Guinea pig This program used 144 guinea pigs and was conducted in the same manner as the mouse experiment with the exception that in this experiment prothrombin determinations and erythrocyte counts were also made.

Aerosol Preparation and Generation

The neodymium used in this experiment was obtained as a pure oxide from the Ames Laboratory of the United States Atomic Energy Commission. It was supplied as a course, irregular, granular material with individual granules varying in diameter from 1 to 15 mm. This product was reduced to a moderately uniform and satisfactory mean particle diameter by two stages of grinding. The first stage was the same as that used in the intratracheal pilot experiment. Here the oxide was pulverized in a Pica Blender mill using stainless steel vials and balls for eight minutes. The powder was reground manually in a mortar with a ceramic pestle and in alcohol. This fluid grinding further reduced the particle size. The alcohol was removed from the mixture by burning, and a satisfactory dry dust remained. This neodymium oxide was stored in an evacuated dessicator until it was used.

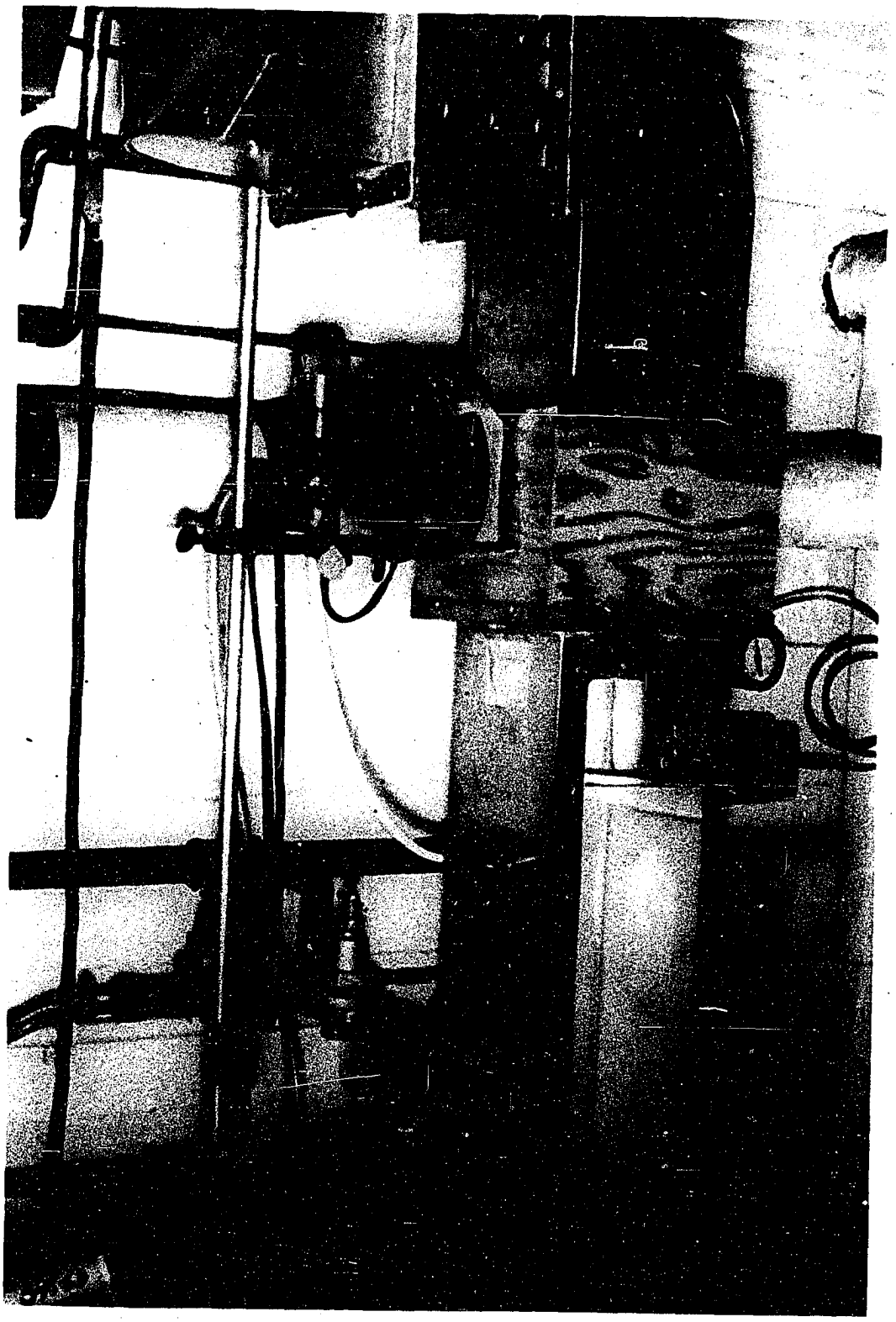
The problem of suspending the aerosol in the air of the inhalation chamber was solved by the use of the Wright Dust Feed Mechanism.[7] The preground dust was tightly packed into a cylindrical holder to form a stable cake. The dust was scraped off the surface by a scraper head.

[7]Purchased from L. Adams, Ltd., London, England.

This head had a groove extending from its center to its edge. The dust was scraped into this groove along which a stream of air was passed so that the dust was carried through the hole in the center of the plate and down an inner tube, through a jet, and against an impaction plate which broke up any remaining aggregates. The scraping was carried out by rotating the dust holder around a threaded spindle through the intermediary of three gears which acted as a differential gear and caused the holder to travel very slowly down the spindle. In this way an extremely thin layer of dust was scraped off at each revolution of the holder (about 0.02 inch). The mechanism was driven by an electric clock motor through a train of change wheels so that a large range of speeds could be obtained at will.

Concentration of aerosol in the chamber was dependent on three factors: the train of change wheels or gears, the air pressure going through the Wright mechanism, and the air flow through the chamber itself. Each of these factors will vary with different materials to be suspended. In the case of neodymium oxide an eighteen toothed gear on the motor spindle, a thirty-six toothed gear on the driven wheels, and a fifty-four toothed gear on the cross shaft coupled with an air pressure of 5 pounds/inch^2 and a chamber through-put air value of 8 ft^3/min. provided the proper dispersal of oxide. Figure 7 shows a small chamber with the dust feed mechanism in place. The dust laden air from the Wright Dust Feed Mechanism was injected into the main stream of chamber air in the throat of the chamber just below the upstream absolute filter. This assured that the aerosol was the only foreign material in the chamber atmosphere. Uniform dispersion of the oxide was excellent as demonstrated by Cook

Figure 7. Wright dust feed mechanism attached to chamber system.



(1961). The aerosol was removed from the atmosphere by the down stream absolute filter.

It was found that dehumidification of the air to the Wright dust feed mechanism was necessary to prevent clogging of the scraper plate. This was accomplished by the use of two filters. The first was a trap at the tank of the central air-compressor unit that served all of the chamber generators. The second was an individual air filter and dehumidifying unit in the line between the solenoid valve on the pressure line and the dust feed mechanism at each chamber unit. These small filters were changed periodically and did an adequate job of supplying dry, clean air to the dust feed units.

Aerosol Characterization

Some of the greatest pitfalls in inhalation toxicity studies lie in the field of the determination of effective dosage. This was critical and difficult to ascertain since it involved both the concentration of the aerosol in the air and its particle size. Both of these parameters directly influenced availability of the material to the animals. At the outset of this experiment it was decided that a concentration in the air of neodymium oxide of 30 mg/meter^3 with the majority of the particles being less than 1 μ in diameter would be ideal.

Concentration in Air

Concentration of neodymium oxide in each chamber being used was determined daily. This was accomplished by drawing a known volume of chamber air across a 1 inch Gelman AM-5 cellulose acetate filter. Each chamber was equipped with a solenoid valve opening to a common vacuum line for all chambers. Vacuum was supplied by a pump, and flow was

gauged by a rotameter. This was set to move 16.8 ft.^{3}/hr. across the filter when the solenoid was opened. The filter paper was held in a stainless, open Gelman filter holder. The holder was loaded with its filter paper, capped to make the unit airtight and suspended from a stainless steel yoke on the wall of the chamber before each day's inhalation run was initiated. During the day the filter cap was removed, solenoid opened for 15 minutes and the sample taken. The solenoid valve was then closed and the holder recapped. At the end of each day the exposed filters were collected for chemical determination of the rare earth impinged on its surface. Manipulations were made possible, without disrupting the chamber atmosphere, by the glove ports in each chamber.

The method described by Fritz et al. (1958) was used to determine the amount of neodymium on each filter paper. This method uses arsenazo 3- (2-arsonophenylazo)-4, 5-dihydroxy -2, 7-naphthalene disulfonic acid for the quantitative colorimetric determination of yttrium and the rare earths. It was applicable to the determination of very low concentrations of rare earth and is not highly pH dependent.

In this experiment the neodymium oxide was eluted from the filter with approximately seven ml of concentrated nitric acid. This was diluted to a total volume of 50 ml. A 1 ml aliquot of this dilution was used for the determination. The sample was buffered with a triethanolamine buffer. Purified arsenazo solution was added and the pH was adjusted to 8.1 with ammonium hydroxide. The resultant color was measured against a reagent blank, prepared with unexposed filter paper, at 570 mμ in a Beckman Model DU spectrophotometer. Results were reported as mg/meter^3 of air. Determinations on 485 daily mouse chamber samples gave

a mean concentration of 29.57 mg. Nd_2O_3/meter^3 of air with a standard deviation of 9.8. Determinations on 510 daily guinea pig chamber samples gave a mean concentration 27.6 mg Nd_2O_3/meter^3 of air with a standard deviation of 9.7. Ninety-nine per cent confidence limits for these means were computed as suggested in the ASTM Manual on Quality Control of Materials (1951) with values of α computed from Table IV, "Table of t", in R. A. Fisher's "Statistical Methods for Research Workers", based on Student's distribution of $\underline{z}$. This calculation ($\bar{x} \pm \alpha\ \sigma$) gave values of 29.57 ± 1.17 for the mice and 27.6 ± 1.06 for the guinea pigs. In view of the many variables concerned, these means were viewed as being remarkably close to those desired. The standard deviation was not of great significance since exposure was over a prolonged period of time and since there was no trend toward higher or lower concentrations at any particular time during the experiment.

Particle Size Determination

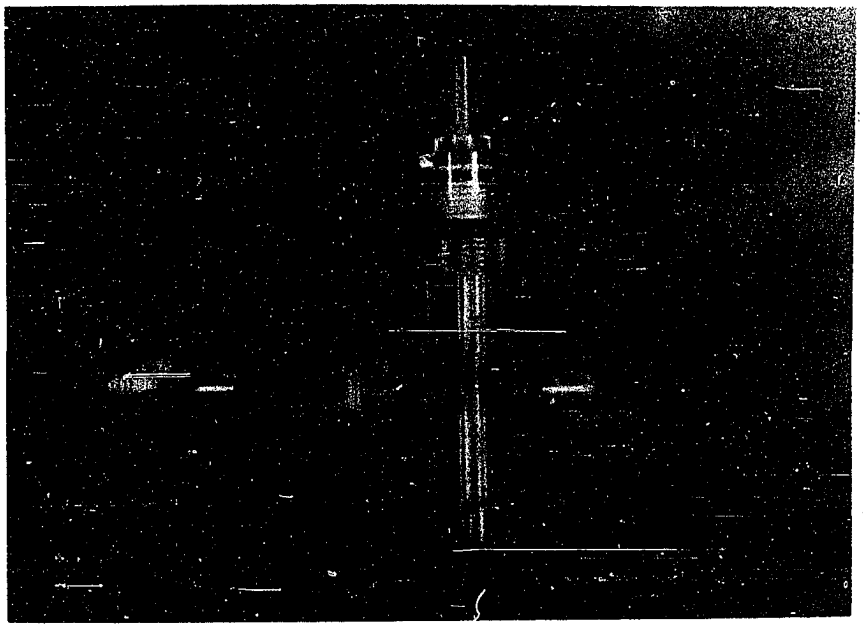
Particle size determination represents a specialized study of its own, and yet it was felt that certain information regarding this parameter would have to be made available if the experiment were to be quantitative.

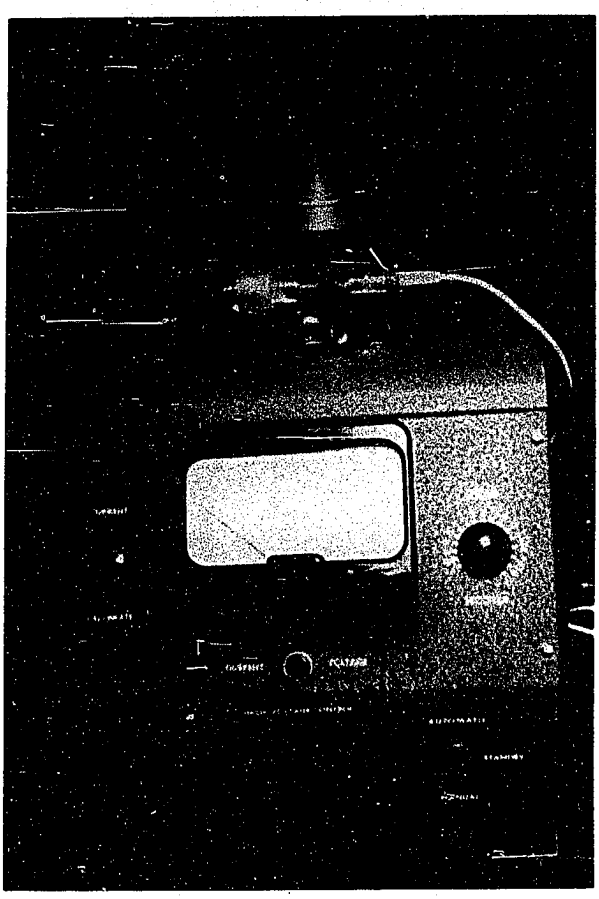
In this experiment attempts have been made to characterize the dust aerosol which was produced according to particle diameter and mass. This characterization was accomplished as described below.

Chamber atmosphere was sampled with the point-to-plane electrostatic precipitator shown in Figures 8 and 9. This instrument was constructed by the Instrument Shop of Iowa State University to specifications provided by Dr. Paul Morrow of the University of Rochester,

Figure 8. Point-to-plane electrostatic precipitator.

Figure 9. Point-to-plane electrostatic precipitator with its power source.





Rochester, New York. The power supply was designed and built by Mr. A. O. Stattelman formerly of Iowa State University.

Samples were precipitated directly on a parlodion coated, athene, 200 mesh, electron microscope grid which had been overlaid with a carbon film. Precipitation was accomplished by drawing chamber air through the instrument at a flow rate of 2.5 $ft.^3/hr.$ using the vacuum pump supplying the chamber system. A voltage of 7 KV producing 7-8 micro ampheres of current across the air gap for 5 second periods was used to collect samples. This process removed a random sample of particles from the chamber air and deposited them on a grid where they could be photographed with the electron microscope. Figures 10 and 11 represent typical plates from such sampling. Particles were then manually measured and counted. Initially all particles were measured along their maximum diameter. However, this procedure was replaced with one in which the maximum diameter horizontal with the bottom or top edge of the electron photomicrograph was recorded. This change was made since volume was also of interest and since maximum diameters overestimated the particle volumes. It was also considered that the site of deposition would not necessarily be determined by the maximum diameter since the particle could just as easily present itself to air passages with its long axis parallel to the passage as with it transverse.

This direct counting gave a mean particle diameter and $\underline{s}$ value of 0.279 ± 0.129 μ. Ninety-nine per cent confidence limits on the mean were $\pm$ .011 μ. Examination of these data showed that the particle diameter distribution was logarithmically normal, and all calculations were based on this type of distribution. These figures represented that portion of

Figure 10. Electron photomicrograph of a typical field of particles precipitated with the electrostatic precipitator. Original picture was taken at 12,500 magnifications.

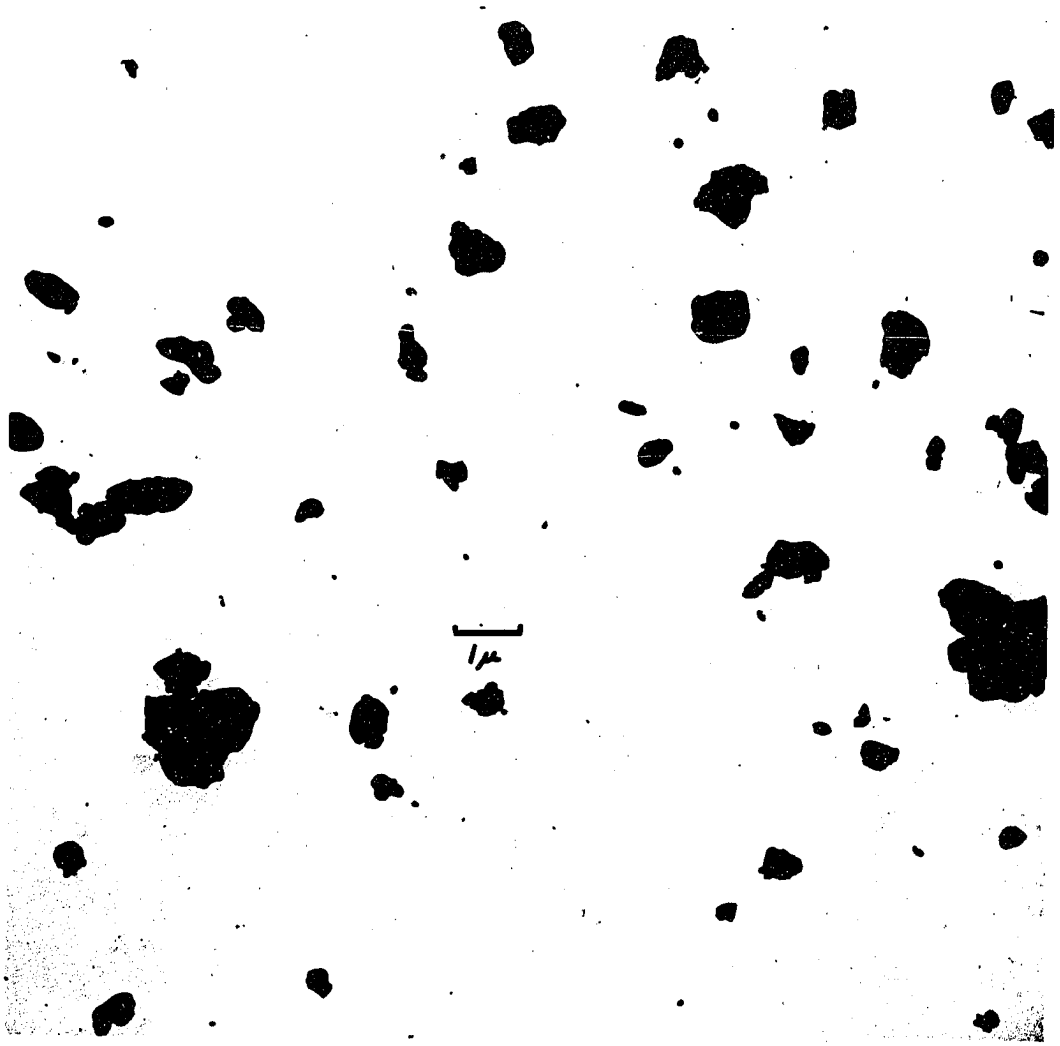

1μ

Figure 11. Electron photomicrograph of a typical field of particles precipitated with the electrostatic precipitator. Original picture was taken at 12,500 magnifications.


1μ

the population that could be seen and measured at 12,500 magnifications. It was observed that electron photomicrographs at 90,000 magnifications still presented particles too small to count. It will be shown later that these extremely small particles made up as much as 20 per cent of the total mass of the aerosol. The effect of these ultra small (less than 0.01 μ) particles on the calculated mean particle diameter can be shown simply by taking electron photomicrographs of the electrostatic precipitate at increasing magnifications and observing that the mean diameter becomes smaller. In this research the mean particle diameter figure was determined at 12,500 magnifications. This was permissible since aging of aerosols, and the humidity and temperature of the respiratory tract should have caused these ultrasmall particles to aggregate and form larger units.

In addition to knowing the appearance of the generated particle it was essential to know what per cent of the total mass was made up of specific particle size ranges.

To accomplish this a Casella Cascade Impactor[8] was employed. This instrument consists of a system of four air jets impinging in series on glass discs. The jets are progressively finer, so that the speed and, therefore, the efficiency of impaction of particles on the discs increases from jet to jet when air is drawn through at a steady rate. The fifth stage is a filter paper between the fourth jet and the line to the vacuum pump. A size grading thereby results which greatly assists assessment of the samples. The jets were designed so that line deposits

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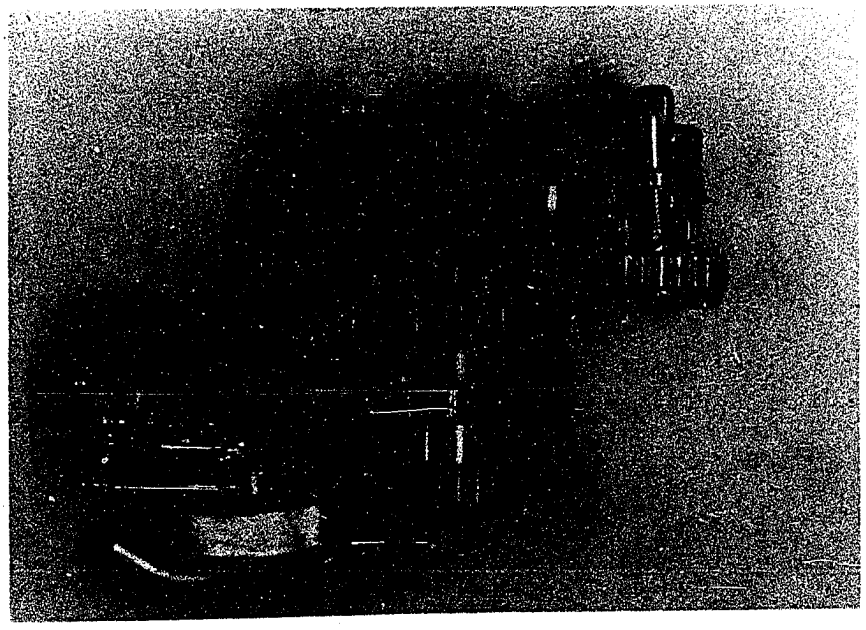
[8]Purchased from Mine Safety Appliance, Pittsburgh, Pennsylvania.

are obtained. The air mover used in this experiment was a Gelman vacuum pump and the flow rate was adjusted to 17.5 liters/minute. This gave velocities through the four jets of 2.2, 10.2, 27.5, and 77 meters per second, respectively. Figures 12 and 13 show this instrument separated from and with its portable vacuum source. The results of 16 determinations at sampling times of 10 and 15 seconds, each from the chamber environment, are given in Table 3. Runs were pooled in order to facilitate chemical analysis for the neodymium impinged at each stage. These figures demonstrated that the per cent of rare earth oxide extracted at each stage was reproducible. Next it was important to ascertain what particle size range was included at each stage of separation.

In order to gather this information, electron microscope grids were mounted in the line of deposition at each disc and particles were impinged directly on these grids for counting. A three second sampling period was found to be optimal for impacting large numbers of particles with the minimum of piling of particles. Typical electron photomicrographs from stages 3 and 4 of the impactor are revealed in Figures 14 and 15. Those particles on stages 1 and 2 were inspected with the optical microscope and found to be relatively large (1.5 μ) in diameter. These particles were few in number. Even though these two stages removed about 25 per cent of the total mass, the size of the particles largely eliminated this portion of the neodymium oxide from that deposited in the alveoli of the test animals. Direct counts on electron photomicrographs of stages 3 and 4 using the same methods as those used to determine the mean particle diameter of the electrostatically precipitated sample gave mean diameters and $\underline{s}$ values for stage 3 of 0.5693 $\mu \pm .344\ \mu$ and

Figure 12. Casella cascade impactor.

Figure 13. Casella cascade impactor with portable vacuum pump.



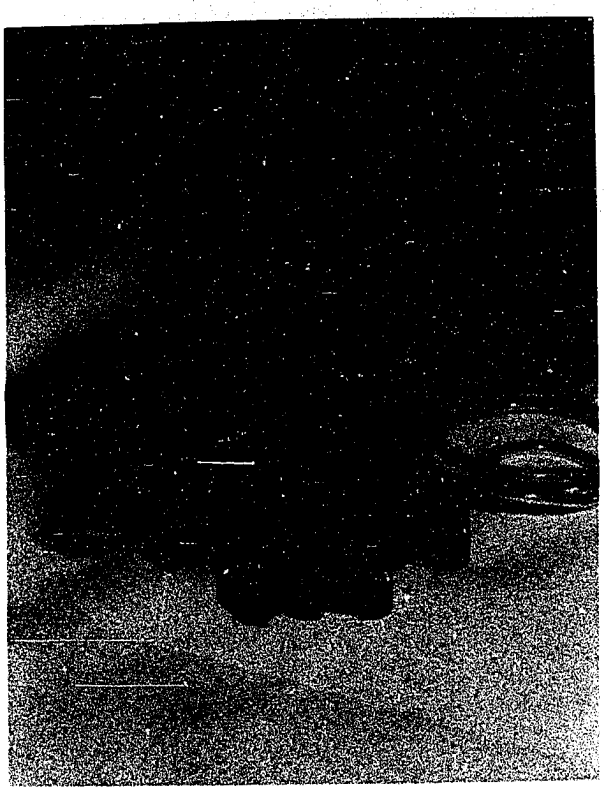


Table 3. Analysis of neodymium oxide fractionation by the casella cascade impactor

Results given in per cent of total mass removed at each stage

Determination	No. of runs pooled for determination	Sampling time each run (sec)	Per cent Nd_2O_3 removed at each stage of the impactor				
			1	2	3	4	5
1	4	10	4	20	30	19	27
2	1 3	10 15	7.2	23.8	31.6	16.5	20.9
3	4	15	2.6	20.5	37.7	17.1	22.1
4	4	15	8.9	17.9	29.2	15.5	28.5
Σx			22.7	82.2	128.5	68.1	98.5
Σx^2			153.8	1707.1	4172.5	1166.0	2466.5
$(\Sigma x)^2/\eta$			128.82	1689.2	4128.0	1159.4	2425.5
$\bar{x}$			5.7	20.6	32.1	17.0	24.6
s^2			8.3	6.0	14.8	2.2	13.6
s			2.8	2.4	3.8	1.5	3.7
$\bar{x} \pm s$			5.7 ± 2.9	20.6 ± 2.4	32.1 ± 3.8	17.0 ± 1.5	24.6 ± 3.7

Figure 14. Electronphotomicrograph from stage three of the Lase
cascade impactor. The original picture was taken a
7,500 magnifications.

Figure 14. Electronphotomicrograph from stage three of the Casella cascade impactor. The original picture was taken at 7,500 magnifications.

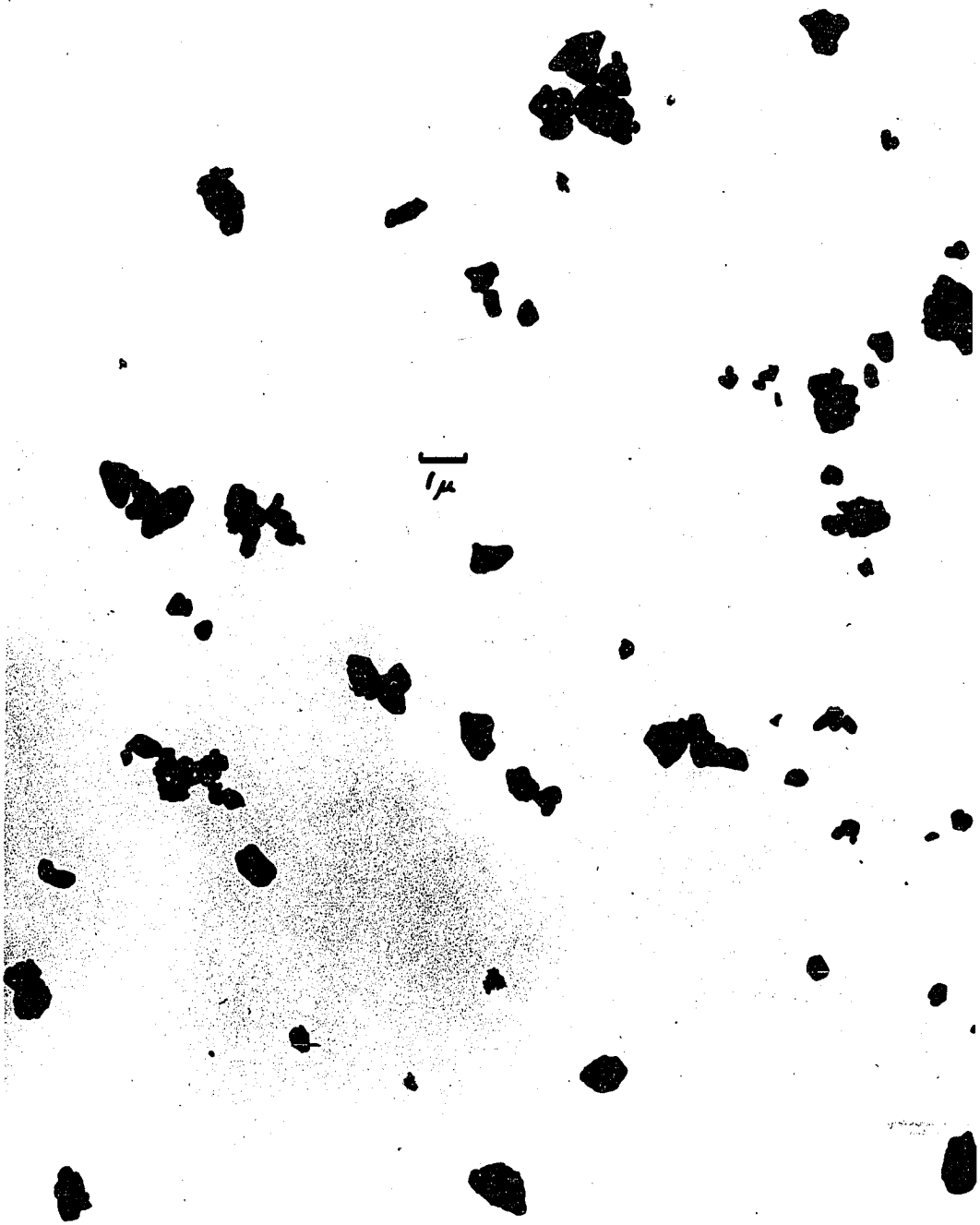

1μ

Figure 15. Electronphotomicrograph from stage four of the Casella cascade impactor. The original picture was taken at 7,500 magnifications.

for stage 4 of 0.346 μ ± .334 μ. Examination of grids from the filter paper (final) stage of the impactor showed particles of diameters well under 0.1 μ with a few aggregates. This stage collected about 20% of the total mass impinged by the impactor. The relationship of particle diameter to mass is a cubic function so that as diameter increases, volume or mass increases at a rapid rate. This fact emphasized the numbers of small particles that were necessary to represent 20 per cent of the total mass on the final stage of the impactor. This fraction of particle numbers decreased as the aerosol ages since the small particles did aggregate.

These data indicated that approximately 60 per cent of the mass of the dust generated was contained in particles less than 1.0 μ in diameter.

In order to arrive at an estimate of dosage the air intake of the experimental animals had to be determined for use with the aerosol concentration and particle size data. These pulmonary data are available in the literature; however, variations in reports were so great that it was decided to derive our own figures from our own colonies.

Oxygen consumption rate determinations were conducted on groups of white mice and guinea pigs representative of those used in the inhalation experiment. This was accomplished by placing the animals in an airtight, oxygen filled container. The carbon dioxide exhaled was absorbed with soda lime and the inside pressure kept normal with room atmospheric pressure by replacing oxygen. This is the system that is used in the teaching laboratories of the Department of Physiology and Pharmacology of the College of Veterinary Medicine of Iowa State University.

The white mice averaged 27.7 grams and had an average oxygen consumption rate of 3.31 ml O_2/gram body weight/hour with a range of 2.67 - 4.05 ml O_2/gram body weight/hour. The determinations were made at 26° C. Fuhrman et al. (1946) reported comparable values in albino mice that were not fasted or anesthetized. He gave a range of 2.15 - 4.08 ml O_2/gram body weight/hour with an average of 3.5 ml O_2/gram body weight/hour.

The oxygen consumption rates for the guinea pigs ranged from .399 - .618 ml/gram body weight/hour with an average value of .505 ml. The weights of guinea pigs ranged from 510 - 921 grams with an average of 612. Determinations were made at 29° C. These values compare favorably with those of Sullivan and Mullen (1954) who recorded a value of 0.35 - 0.39 ml O_2/gram body weight/hour for guinea pigs averaging 772 grams at 25 - 35° C. Morrison (1948) also listed metabolic rates which, when converted back to oxygen consumption rates, gave a range of 0.57 - 0.77 ml O_2/gram body weight/hour for guinea pigs weighing 410 - 590 grams.

Dose Calculation

With the preceeding information an idea of dosage was established following a model similar to the so-called Harriman Conference model presented in the recommendations of the International Commission of Radiological Protection for 1955. This stated that the average mouse breathed 1980 ml of air per hour. This value was derived from the oxygen consumption studies carried out on mice. There was an average of 30 mg Nd_2O_3/meter^3 of air available. Each mouse breathed about 0.119 meter^3 of this air during each 10 day period of exposure; and, based on the particle size-mass relationship, it was estimated that 60 per cent of this dust was of a character that would permit deposition in the

terminal bronchioles and alveoli. From these figures dosages could be estimated which gave an index of the amount of material to which each mouse was being exposed. The same process is possible for guinea pigs and the values derived for both mice and guinea pigs are given in Tables 4 and 5. The assumption of 100 per cent retention was incorrect if lung burden alone is considered. If this material were translocated, it still contributed to the total body burden of neodymium. This assumed no excretion.

Hematologic Studies

Hematologic examinations consisted of hemoglobin, packed cell volume (PCV), coagulation time, and total and differential leucocytes counts in mice and guinea pigs. Erythrocyte enumeration and prothrombin determination were also accomplished for the guinea pigs.

Erythrocyte and leucocyte enumerations were determined in duplicate, utilizing National Bureau of Standards certified pipettes, counting chambers, and cover glasses. Acceptable counts differed no more than twice the square root of the mean of the highest and lowest counts obtained in each counting square of the hemacytometer chamber. The diluting fluids used were Hayem's solution for erythrocytes and 0.1 N HCl for leucocytes.

The PCV was determined by the microhematocrit capillary tube method. Capillary tubes were filled with fresh oxalated blood at the time of bleeding and centrifuged for 5 minutes at 10,000 r.p.m.

Hemoglobin concentrations were determined by the acid hematin method of Cohen and Smith (1919), utilizing 20 cmm. of whole blood and 5 ml of 0.1 N HCl. After allowing 30 minutes for color development, samples were read in a Coleman Junior spectrophotometer against a 0.1 N HCl

Table 4. Estimated dosage of inhaled neodymium oxide in Rockland albino mice

Based on an air consumption of 1980 ml/hour
or 11,880 ml air/6 hour exposure day

Days Exposed	Meters^3 air breathed	Mg Nd_2O_3 available in air breathed *	Mg Nd_2O_3 available to lung **	Estimated dose mg/kg body wt. ***
10	.119	3.56	2.14	77.26
20	.238	7.13	4.28	154.51
30	.356	10.69	6.41	231.77
40	.475	14.26	8.56	309.02
50	.594	17.82	10.69	386.92
60	.713	21.38	12.83	463.18
70	.832	24.95	14.97	540.43
80	.950	28.51	17.11	617.69
90	1.069	32.08	19.25	694.95
100	1.188	35.64	21.38	771.84
110	1.307	39.20	23.52	849.10
120	1.426	42.77	25.66	926.35

* based on 30 mg Nd_2O_3/m^3 of air
** based on 60% availability of total mass due to particle size distribution
*** based on assumption of 100% retention

Table 5. Estimated dosage of inhaled neodymium oxide in guinea pigs

Based on an air consumption of 6676 ml/hour
or 40,056 ml air/6 hour exposure day

Days Exposed	Meters^3 air breathed	Mg Nd_2O_3 available in air breathed *	Mg Nd_2O_3 available to lung **	Estimated dose mg/kg body wt.***
10	.406	11.37	6.82	11.14
20	.811	22.74	13.64	22.29
30	1.217	34.10	20.47	33.43
40	1.622	45.47	27.29	44.58
50	2.028	56.84	34.11	55.72
60	2.434	68.21	40.93	66.86
70	2.839	79.58	47.75	78.01
80	3.245	90.94	54.58	89.15
90	3.650	102.31	61.40	100.29
100	4.056	113.68	68.22	111.44
110	4.462	125.05	75.04	122.58
120	4.867	136.42	81.86	133.73

* based on 28 mg Nd_2O_3/m^3 of air
** based on 60% availability of total mass due to particle size distribution
*** based on assumption of 100% retention

blank. Standards were prepared for calibration of the spectrophotometer from a blood sample of known hemoglobin content determined by the iron method of Wong (1928).

Coagulation time was determined by the capillary tube method as cited by Gradwohl (1956).

Prothrombin determinations were carried out by using the one step method of Quick (1940).

Weights

All animals were weighed weekly. The mice were weighed in groups of ten and average weights used for individuals. The guinea pigs were weighed individually.

Macroscopic Studies

Necropsy examinations were performed on all animals.

Microscopic Studies

Portions of sternum, lungs, heart, liver, spleen, adrenals, kidneys, tracheo-bronchial and mesenteric lymph nodes and testes or ovaries were taken for histopathologic examination.

These tissues were fixed in 10 per cent formalin. Paraffin sections were cut at 6 microns and stained with hematoxylin-eosin. Many were also stained by Gomori's one step trichrome method.

RESULTS

Hematologic Findings

Hemoglobin values for each of the twelve mice and twelve guinea pigs were averaged for each 10 day time period. These mean values are plotted in Figure 17 and Figure 21. They ranged from 12.3 to 16.2 gm/100 ml for mice and from 13.4 to 15.4 gm/100 ml for guinea pigs. No significant variations from control values were seen in either species exposed to neodymium oxide.

Packed cell volumes were averaged for each 10 day period for the six control and six exposed animals in both the mice and guinea pigs. These mean values are compared in Figure 16 and Figure 20. They ranged from 39.3 to 53.6 per cent for mice and from 41.0 to 45.7 per cent for guinea pigs. No significant variation from control values was seen in either species.

White blood cell count comparisons with control values are given in Figure 19 for the mice and in Figure 23 for the guinea pigs. Mean values were used for each 10 day interval. Even though variations from 7,467 to 15,752 for mice and 5,483 to 13,366 for guinea pigs were present there were no significant differences between control and exposure values.

White blood cell differential counts are given in tabular form in Table 6 for the mice and in Table 7 for the guinea pigs. The numbers used are means for each 10 day period. These values did not vary significantly between control and exposure groups of animals.

Prothrombin times were determined for the guinea pigs only. These values were not established for the mice since the method used was not

a micro-method. The volume of blood necessary was the limiting factor. Values for guinea pigs which were exposed to neodymium oxide are compared to control groups in Figure 24. Ranges were from 20.1 to 35.2 seconds. No significant differences resulted in this parameter as a result of the exposure.

Clotting time comparisons for exposure and control groups of guinea pigs and mice were made (Figures 18 and 22). These are mean values for each of the groups. They ranged from 2.4 to 4.5 minutes for mice and from 2.4 to 4.4 minutes for guinea pigs. Exposure and control groups did not differ significantly.

Red blood cell counts were determined for the guinea pig groups only. The mean values are given in Figure 25 and showed no significant difference when data from exposure animals were compared to controls. Values ranged from 4.74 to 5.53 million.

Weight Findings

Data on weights were plotted as mean weights for males and mean weights for females at each 10 day period. Male and female weights were not pooled since there is a significant difference between them at maturity. These values are given in Figure 26 for the mice and in Figure 27 for the guinea pigs.

Autopsy Findings

At the end of the 120 day exposure period the lungs from both the mice and the guinea pigs appeared to be normally inflated and exhibited the following evidence of pulmonary damage or change.

Three of the six mice killed at the end of 20 days' exposure exhibited macroscopic lesions of pneumonia in varying stages. Gross

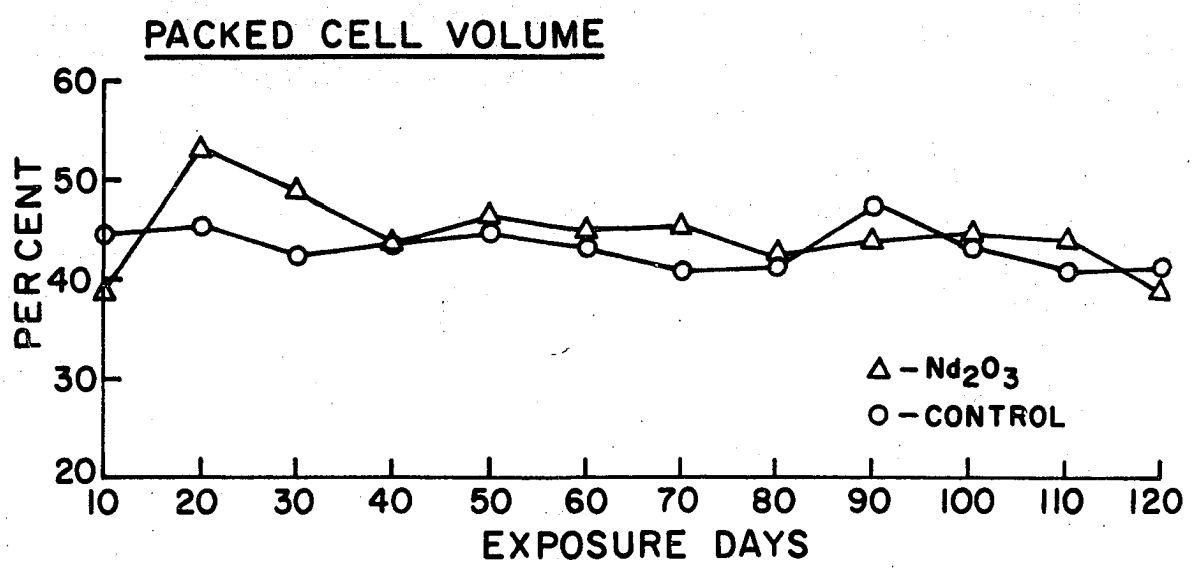


Figure 16. Mice

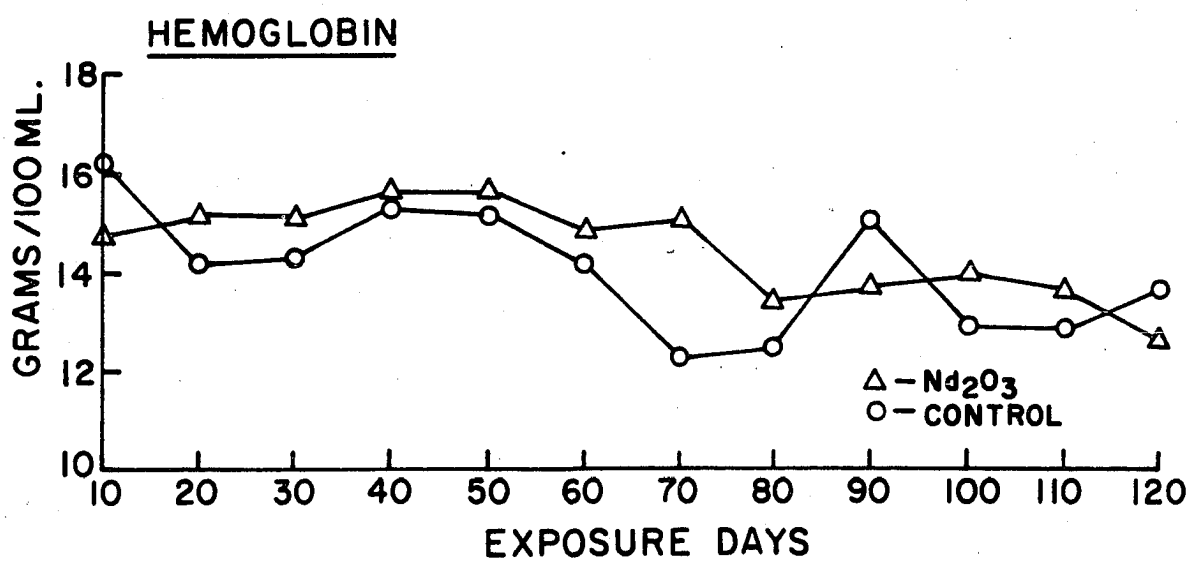


Figure 17. Mice

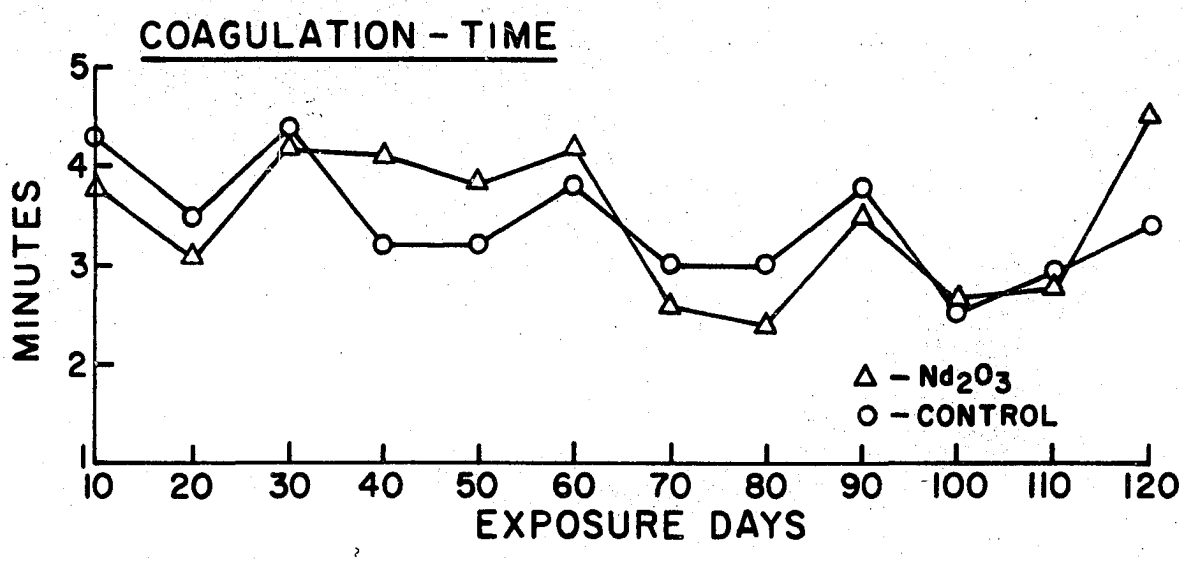


Figure 18. Mice

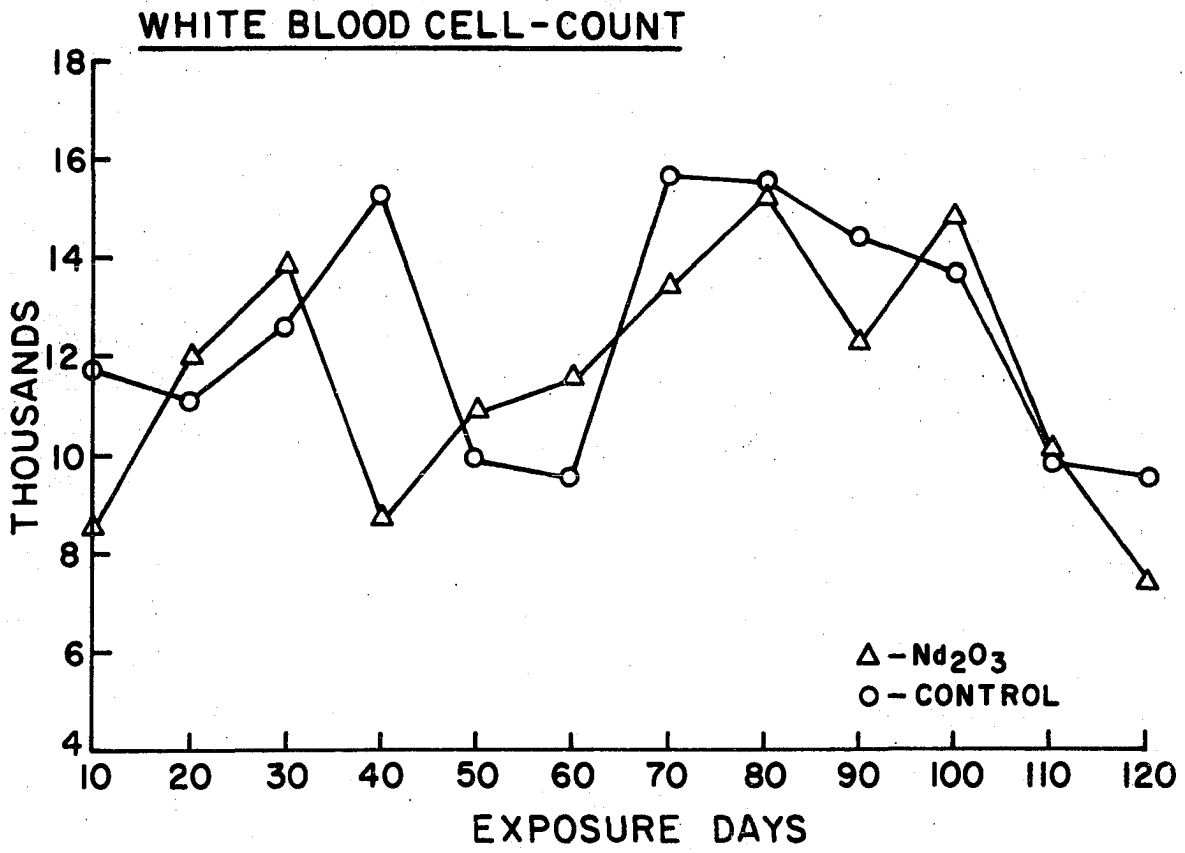


Figure 19. Mice

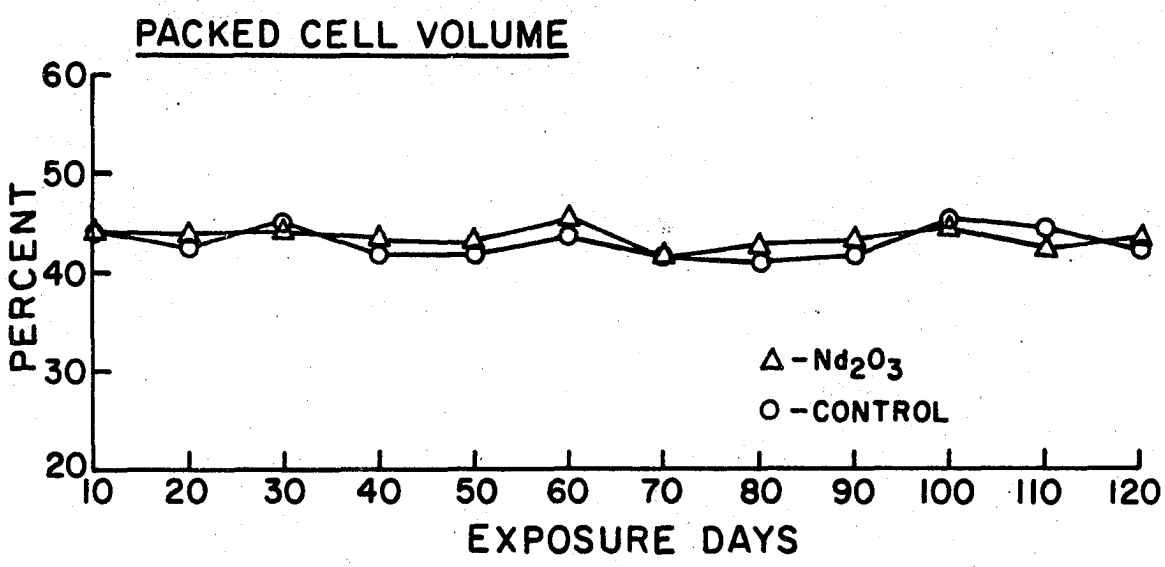


Figure 20. Guinea pig

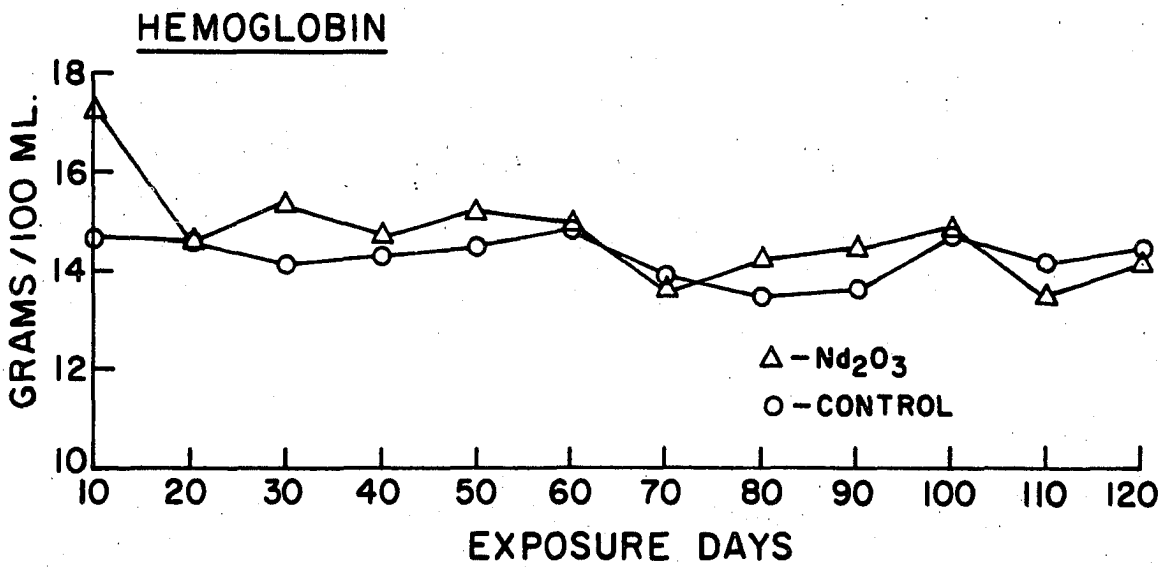


Figure 21. Guinea pig

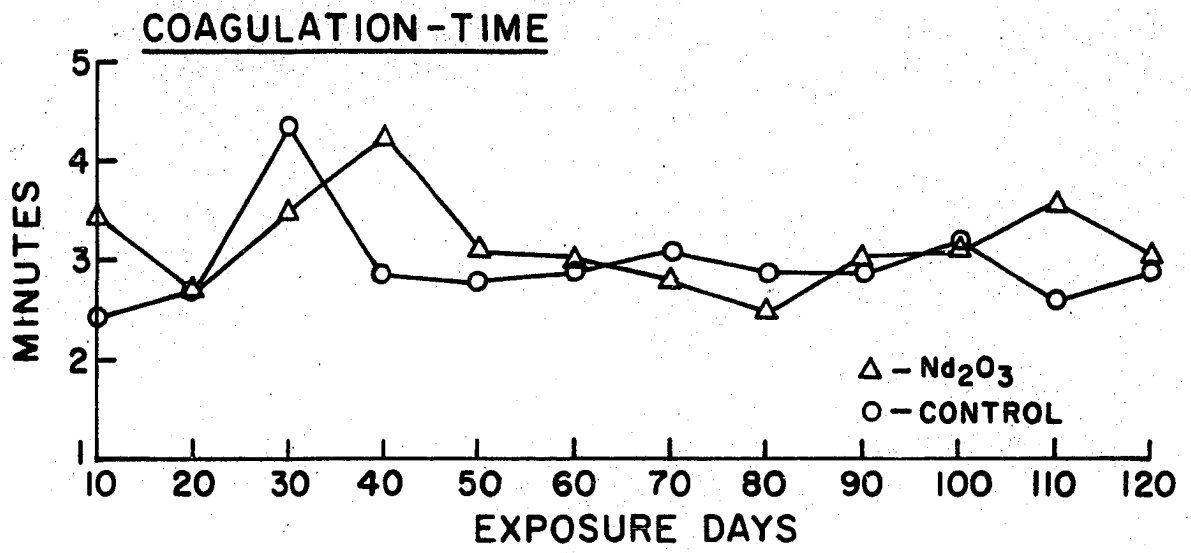


Figure 22. Guinea pig

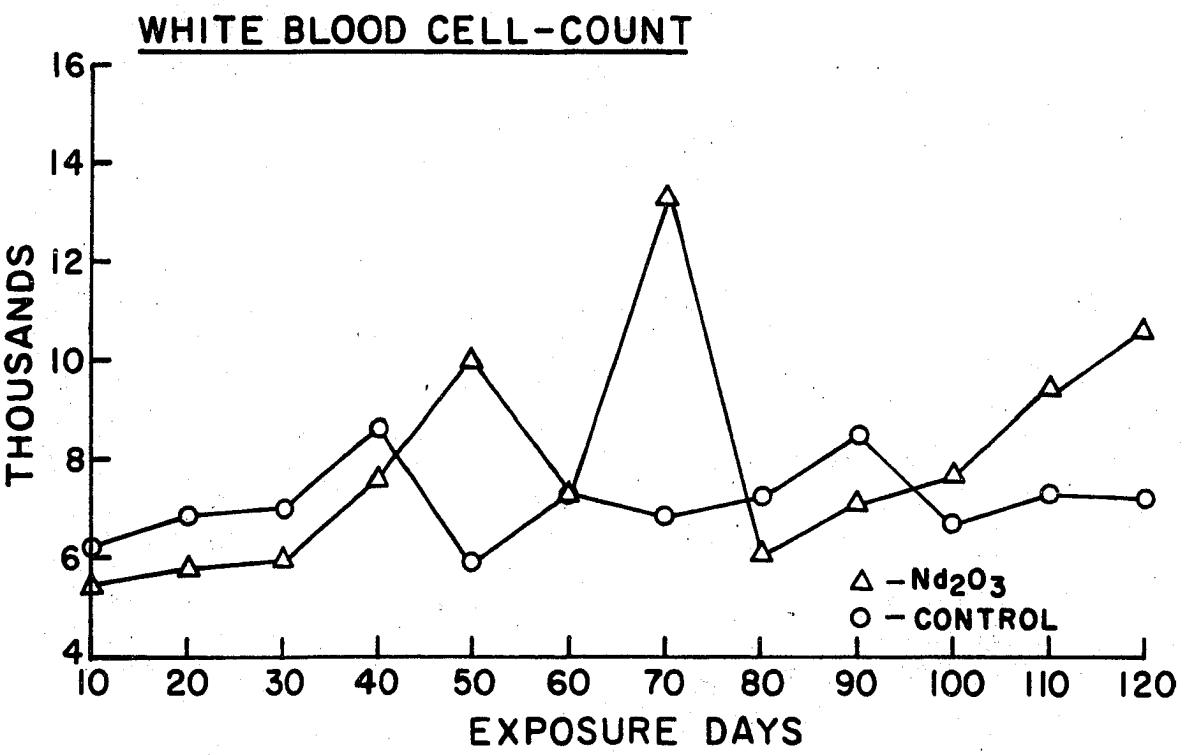


Figure 23. Guinea pig

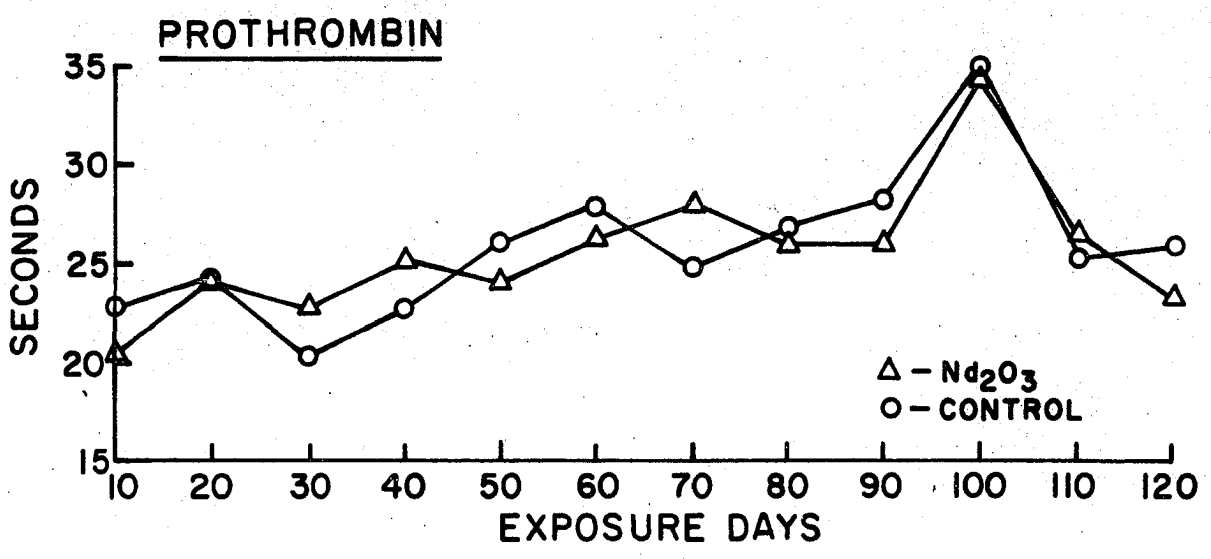


Figure 24. Guinea pig

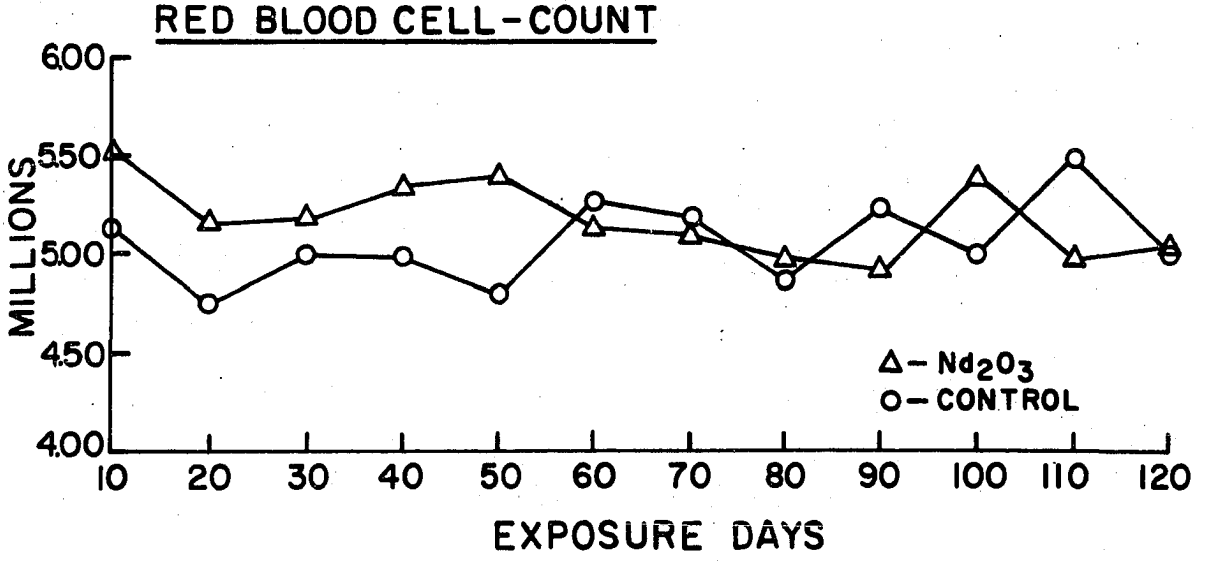
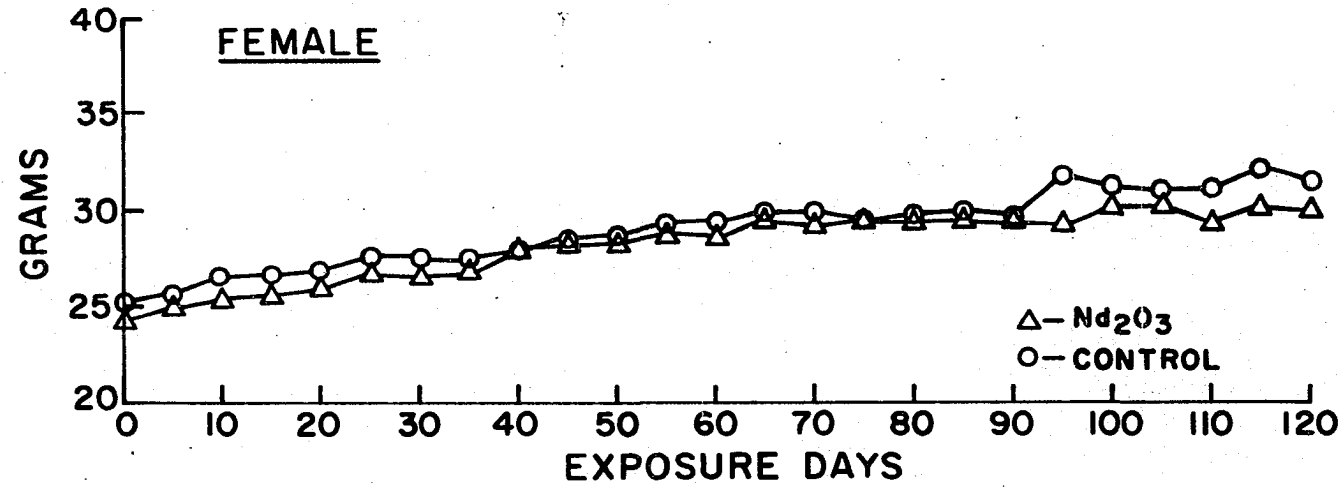


Figure 25. Guinea pig



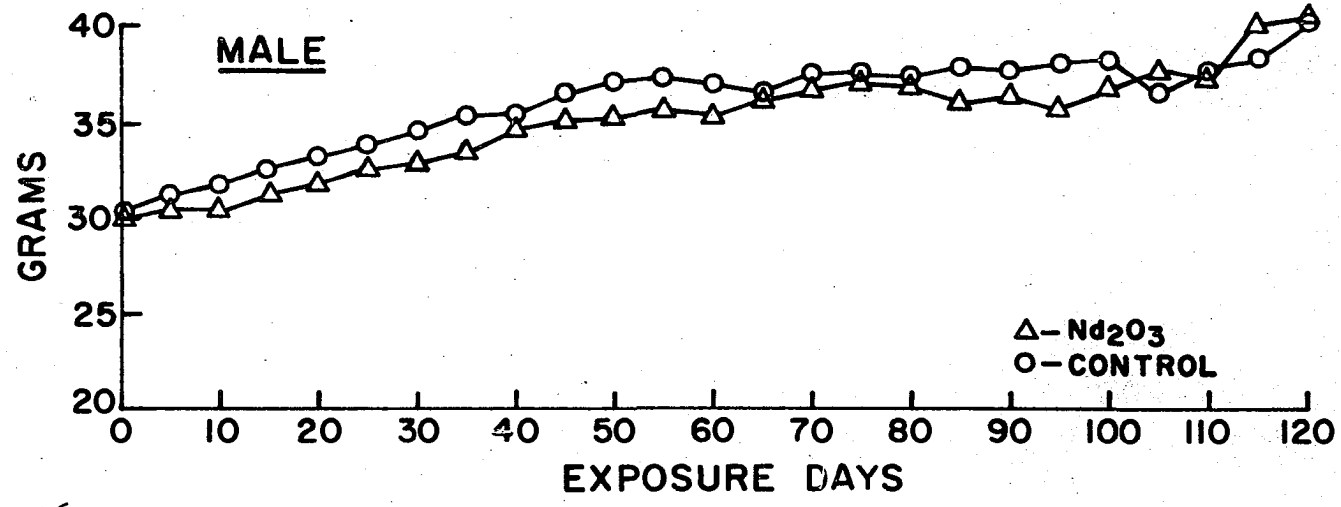
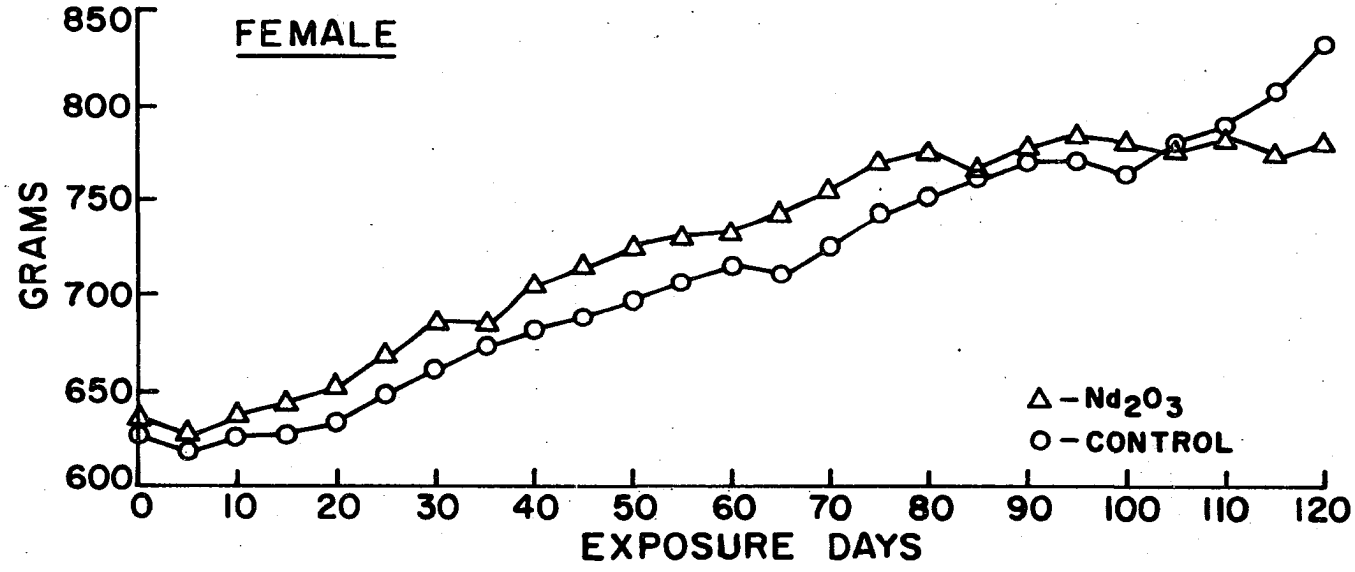


Figure 26. Mice



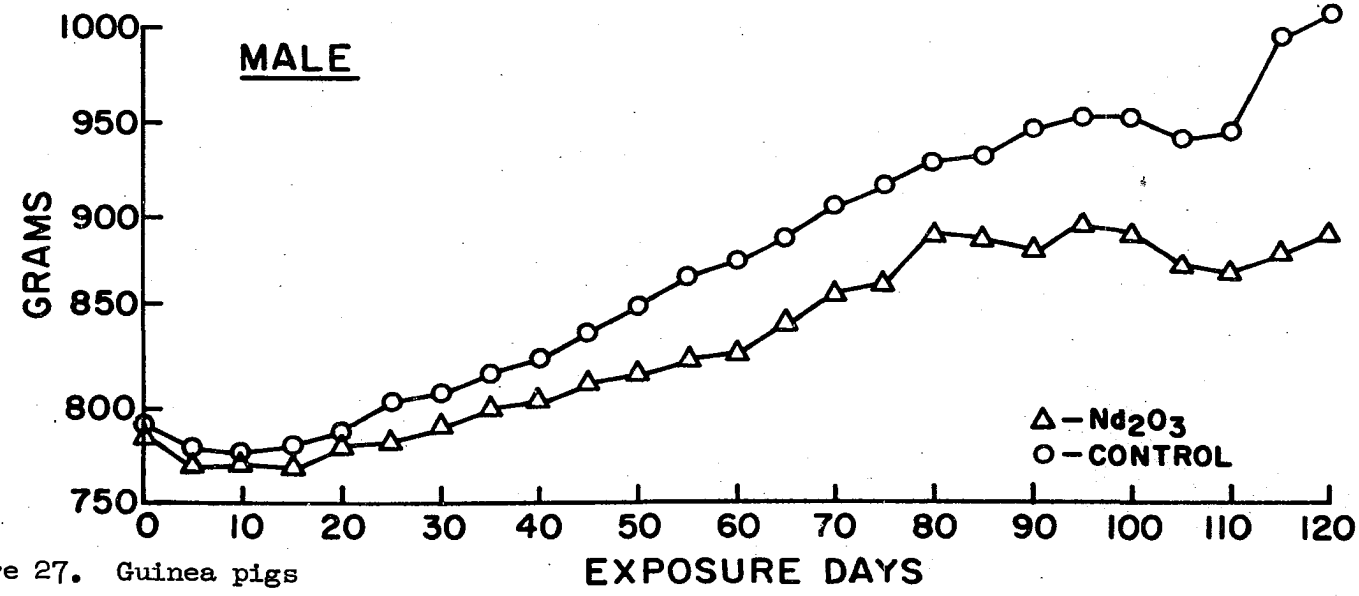


Figure 27. Guinea pigs

Table 6. Differential leucocyte values

Mice

	EXPOSURE					CONTROL				
	Neut.	Band.	Lymph.	Mono.	Eosin.	Neut.	Band.	Lymph.	Mono.	Eosin.
10 day										
male	52.3	7.0	39.0	1.3	0.3	34.7	5.0	54.7	2.0	3.7
female	35.3	4.7	57.3	1.7	1.0	29.0	2.5	66.5	1.5	0.5
mean	43.8	5.9	48.2	1.5	0.7	31.9	3.8	60.6	1.8	2.1
20 day										
male	60.0	3.0	31.3	3.0	2.7	56.7	2.3	35.7	1.3	4.0
female	32.5	5.5	58.5	3.0	0.5	42.3	3.3	50.7	2.0	1.7
mean	46.3	4.3	44.9	3.0	1.6	49.0	2.8	43.2	1.7	2.9
30 day										
male	39.0	6.7	48.7	3.7	2.0	34.0	6.0	55.3	4.0	0.7
female	38.7	6.3	49.3	1.7	4.0	50.7	8.3	36.0	3.0	2.0
mean	38.9	6.5	49.0	2.7	3.0	42.4	7.2	45.7	3.5	1.4
40 day										
male	40.0	3.7	52.0	2.7	1.0	49.3	6.0	41.0	1.7	1.7
female	16.0	3.7	74.7	3.7	2.0	27.7	4.7	64.0	2.7	1.0
mean	28.0	3.7	63.4	3.2	1.5	38.5	5.4	52.5	2.2	1.4
50 day										
male	44.7	6.0	42.0	2.7	4.7	37.0	5.5	53.5	0.5	3.5
female	23.3	4.7	65.3	1.7	5.0	31.0	3.7	58.3	3.7	3.3
mean	34.0	5.4	53.7	2.2	4.9	34.0	4.6	55.9	2.1	3.4
60 day										
male	52.0	2.7	37.7	1.7	2.7	62.0	4.0	28.7	1.0	4.3
female	31.3	5.7	53.3	3.0	6.7	30.7	5.3	57.0	4.0	3.0
mean	41.7	4.2	45.5	2.4	4.7	46.4	4.7	42.9	2.5	3.7

Table 6 (Continued)

	EXPOSURE					CONTROL				
	Neut.	Band.	Lymph.	Mono.	Eosin.	Neut.	Band.	Lymph.	Mono.	Eosin.
70 day										
male	30.7	6.3	59.0	1.3	2.7	45.5	11.0	36.5	4.0	3.0
female	33.5	2.5	62.0	1.0	1.0	26.3	4.7	67.0	1.3	0.7
mean	32.1	4.4	60.5	1.2	1.9	36.9	7.9	51.8	2.7	1.9
80 day										
male	46.3	5.3	43.3	3.3	1.7	51.7	10.7	34.3	2.3	1.0
female	18.7	4.3	74.3	1.7	1.0	17.3	7.0	71.0	2.3	2.3
mean	32.5	4.8	58.8	2.5	1.4	34.5	8.9	52.7	2.3	1.7
90 day										
male	63.0	7.3	28.0	1.7		66.3	4.0	27.7	1.7	0.3
female	32.3	5.0	57.3	3.0	2.3	39.0	7.7	51.7	1.3	0.3
mean	47.7	6.2	42.7	2.4	1.2	52.7	5.9	39.7	1.5	0.3
100 day										
male	51.0	8.3	36.0	3.0	1.7	46.0	7.0	43.0	3.3	0.7
female	28.7	6.7	59.7	1.7	3.3	24.0	4.0	66.7	1.7	3.7
mean	39.9	7.5	47.9	2.4	2.5	35.0	5.5	54.9	2.5	2.2
110 day										
male	52.0	5.7	40.7	1.3	0.3	42.3	5.0	47.3	2.7	2.7
female	35.3	5.3	56.7	2.7		31.7	4.0	63.7	0.7	
mean	43.7	5.5	48.7	2.0	0.2	37.0	4.5	55.5	1.7	1.4
120 day										
male	49.3	10.0	37.0	3.3	0.3	43.0	4.0	49.7	3.0	0.3
female	43.3	3.7	47.3	4.3	1.3	28.0	4.7	65.0	2.3	
mean	46.3	6.9	42.2	3.8	0.8	35.5	4.4	57.4	2.7	0.2

Table 7. Differential leucocyte values

Guinea Pig

	EXPOSURE						CONTROL					
	Neut.	Band.	Lymph.	Mono.	Eosin.	Baso.	Neut.	Band.	Lymph.	Mono.	Eosin.	Baso.
10 day												
male	34.0	0	62.5	2.0	1.0	1.0	38.7	2.0	55.3	3.3	0.7	
female	24.3	1.7	67.7	5.3	1.0		29.3	1.0	56.3	4.0	9.3	
mean	29.2	0.9	65.1	3.7	1.0	0.5	34.0	1.5	55.8	3.7	5.0	
20 day												
male	30.0	1.0	64.5	4.0	0.5		30.5	2.5	63.5	2.0	1.5	
female	10.0	1.0	87.7	0.7	0.3	0.3	6.0	2.0	89.5	1.5	1.0	
mean	20.0	1.0	76.1	2.4	0.4	0.2	18.3	2.3	76.5	1.8	1.3	
30 day												
male	29.3	0.7	63.7	5.3	0.7	0.3	13.0	1.3	83.7	2.0	0	
female	17.7	0	78.3	1.7	2.0	0.3	19.7	1.7	73.0	3.0	2.7	
mean	23.5	0.4	71.0	3.5	1.4	0.3	16.4	1.5	78.3	2.5	1.4	
40 day												
male	36.0	0.3	56.0	4.3	3.3		32.0	2.3	64.3	1.0	0.3	
female	17.0	0.7	77.0	4.3	0.7		5.3	1.7	88.7	3.7	0.7	
mean	26.5	0.5	66.5	4.3	1.7		18.7	2.0	76.5	2.4	0.5	
50 day												
male	29.5	1.5	60.5	4.0	4.5		12.7	0.7	84.7	2.0	0	
female	16.3	1.7	76.7	3.0	1.3	0.3	12.0	3.0	82.0	2.0	1.0	
mean	22.9	1.6	68.6	3.5	2.9	0.2	12.4	1.9	83.3	2.0	0.5	
60 day												
male	32.7	1.7	63.0	2.3	0.3		24.5	1.5	65.5	2.5	6.0	
female	16.7	4.3	75.3	2.7	1.0		11.0	1.3	83.7	2.3	1.7	
mean	24.7	3.0	69.2	2.5	0.7		17.8	1.4	74.6	2.4	3.9	

Table 7 (Continued)

	EXPOSURE						CONTROL					
	Neut.	Band.	Lymph.	Mono.	Eosin.	Baso.	Neut.	Band.	Lymph.	Mono.	Eosin.	Baso.
70 day												
male	31.0	4.5	58.5	6.0			19.7	2.0	73.7	2.7	2.0	
female	17.7	1.0	76.0	2.7	2.3	0.3	14.0	2.0	80.7	2.3	1.0	
mean	24.4	2.8	67.2	4.4	1.2	0.2	16.9	2.0	77.2	2.5	1.5	
80 day												
male	26.7	1.0	70.0	1.7	0.7		17.7	0.3	80.0	1.0	1.0	
female	10.7	2.3	85.3	0.7	1.0		18.3	2.3	72.0	2.3	5.0	
mean	18.7	1.7	77.6	1.2	0.9		18.0	1.3	76.0	1.7	3.0	
90 day												
male	21.0	1.7	74.7	2.3	0.7		16.3	1.3	76.0	4.7	1.7	
female	25.3	3.3	66.3	4.3	0.7		10.0	1.0	85.7	1.0	2.7	
mean	23.2	2.5	70.5	3.3	0.7		13.2	1.2	80.8	2.9	2.2	
100 day												
male	21.3	6.3	70.0	2.3	3.3		24.0	0.7	65.7	3.7	6.0	
female	24.3	2.3	63.3	5.7	4.3		9.3	1.7	81.3	3.3	3.7	
mean	22.8	4.3	66.7	4.0	3.8		16.7	1.2	73.5	3.5	4.9	
110 day												
male	41.3	4.7	49.3	1.3	3.3		16.0	3.0	76.0	4.5	0.5	
female	14.0	3.5	81.5	1.0			12.7	2.0	80.3	1.7	3.3	
mean	27.7	4.1	65.4	1.2	1.7		14.4	2.5	78.2	3.1	1.9	
120 day												
male	24.3	1.7	66.0	4.0	4.0		26.0	2.3	59.0	4.7	7.7	0.3
female	25.0	4.7	64.3	1.7	4.3		16.3	0.7	77.7	5.0	0.3	
mean	24.7	3.2	65.2	2.9	4.2		21.2	1.5	68.4	4.9	4.0	0.2

evidence of pneumonia was also seen in one mouse killed at 30 days, in two mice killed at 50 days and in one killed at 60 days. In mice killed from 60 to 120 days the lungs became increasingly gray in color and at 100, 110, and 120 days petechiations were seen. One mouse in the 120 day group was found to have pleural adhesions to the diaphragm. Tracheobronchial lymph nodes were moderately enlarged and firm after 90 days of exposure.

All of the pulmonary changes mentioned above in the mice were focal and none of the animals killed showed clinical symptoms of illness. Control mice showed no gross pulmonary changes and in all animals, exposure and control, the other organs and glands examined appeared normal.

Lungs from guinea pigs showed a gray discoloration and firmness after 50 days of exposure. These findings were not consistent in all animals killed even at 120 days. Tracheobronchial lymph nodes were moderately enlarged after 70 days of exposure. All lungs, from exposure and control animals, showed some focal areas of atelectasis.

Control animals demonstrated no remarkable macroscopic pulmonary changes. Other glands and organs examined in all guinea pigs appeared normal with the exception of two 10 day control animals which had multiple hepatic and splenic abscesses. Salmonella enteritidis was isolated from these lesions. Two control animals were also found to have a cystic nephritis.

Histological Changes

Lungs of mice exposed to neodymium oxide progressed through an orderly sequence of changes as the exposure time increased.

Initial changes were seen as a slight thickening of the septa with

a mild swelling and proliferation of septal cells. Occasional macrophages were seen containing neodymium at the end of the first 10 days (Figures 28 and 29).

The neodymium seen in the cells appeared as an amorphous material. It was probably a mixture of the oxide, the hydroxide and the carbonate. This is likely since the oxide can be readily converted to the hydroxide under physiological conditions and the hydroxide also combines easily with carbon dioxide to produce the carbonate. All of these forms are relatively insoluble at body pH.

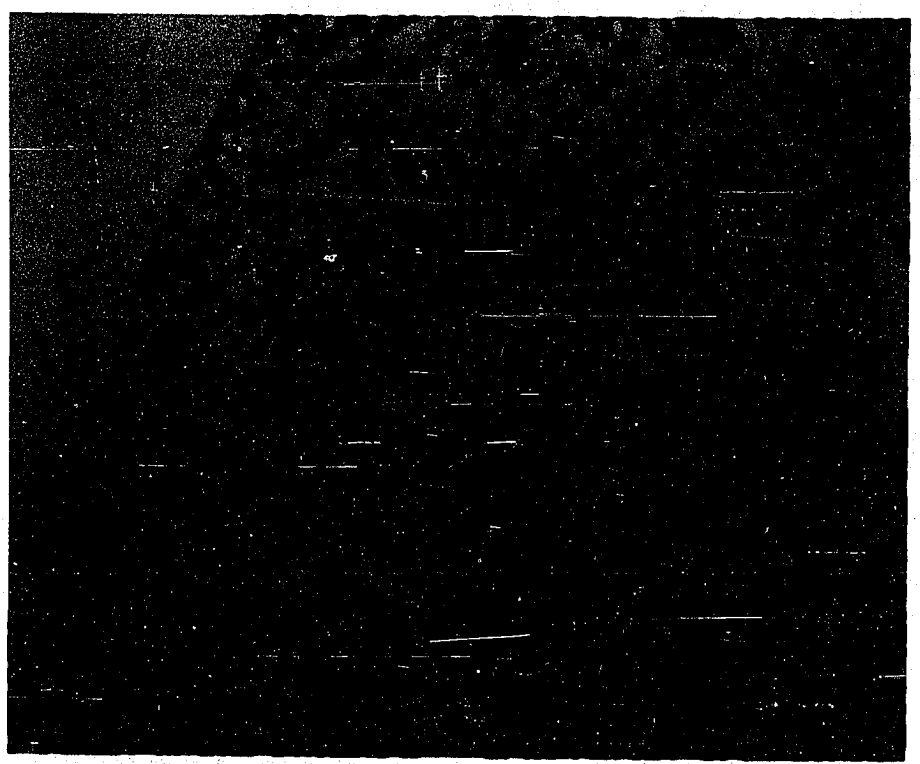
These changes progressed, as seen in Figures 30 and 31, until the septa were thickened to three or more cells in isolated areas and the dependent portions of the lungs contained many macrophages laden with neodymium. Some of these cells were bi- and trinucleated.

The pneumonia diagnosed grossly was verified by microscopic examination in the 20 and 30 day exposure mice (Figures 32 and 33). These lesions were focal and did not resemble the typical lesions of a bronchopneumonia. They were different in that the primary sites were not in the most dependent or ventral parts of the apical, cardiac, or diaphragmatic lobes. It did not show a general progession dorsally and posteriorly but was primarily focal or patchy in distribution. Areas of reaction were seen to contain macrophages packed with neodymium.

The general pattern of impaction of the dust in the lungs with its subsequent phagocytosis by macrophages continued until at 70 days as much as 30 per cent of the functioning tissue appeared to be filled with these rare earth laden cells (Figures 34 and 35). During this period a gradual peribronchiolar lymphocytic hyperplasia occurred in many animals.

Figure 28. Lung from a mouse exposed for 10 days to an aerosol of neodymium oxide. x 67. H. and E. stain.

Figure 29. Lung from a mouse exposed for 10 days to an aerosol of neodymium oxide. Arrows point to the occasional macrophages which were seen containing neodymium. x 450. H. and E. stain.



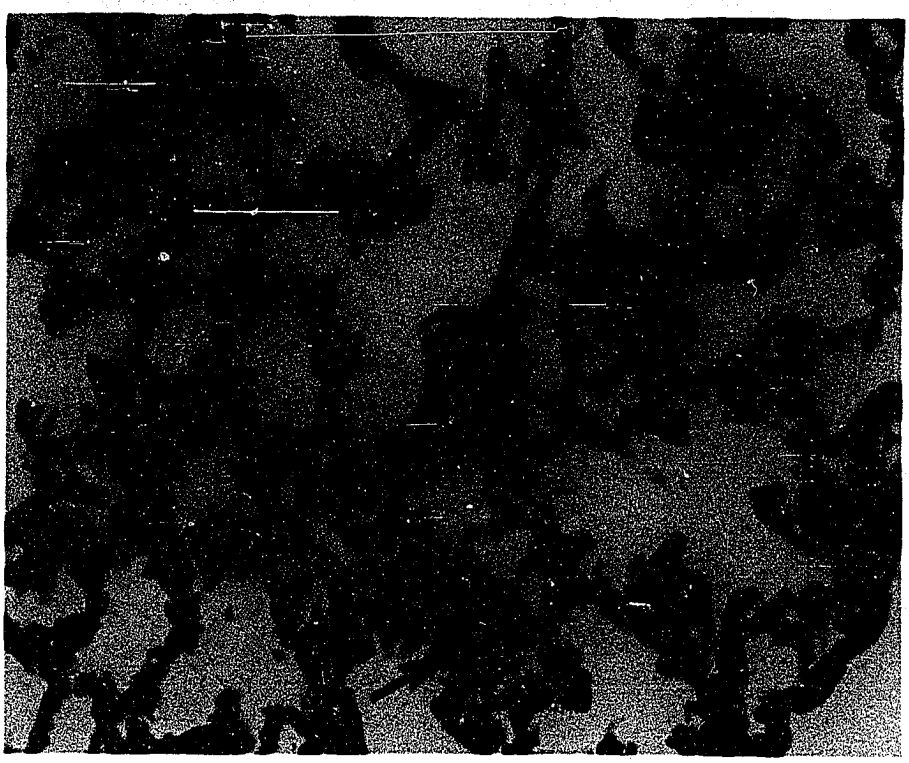


Figure 30. Lung from a mouse exposed for 20 days to an aerosol of neodymium oxide. Thickened septa are evident and macrophages are prominant. x 450. H. and E. stain.

Figure 31. Same lung as shown in Figure 30. Arrows point to macrophages laden with neodymium. x 450. H. and E. stain.

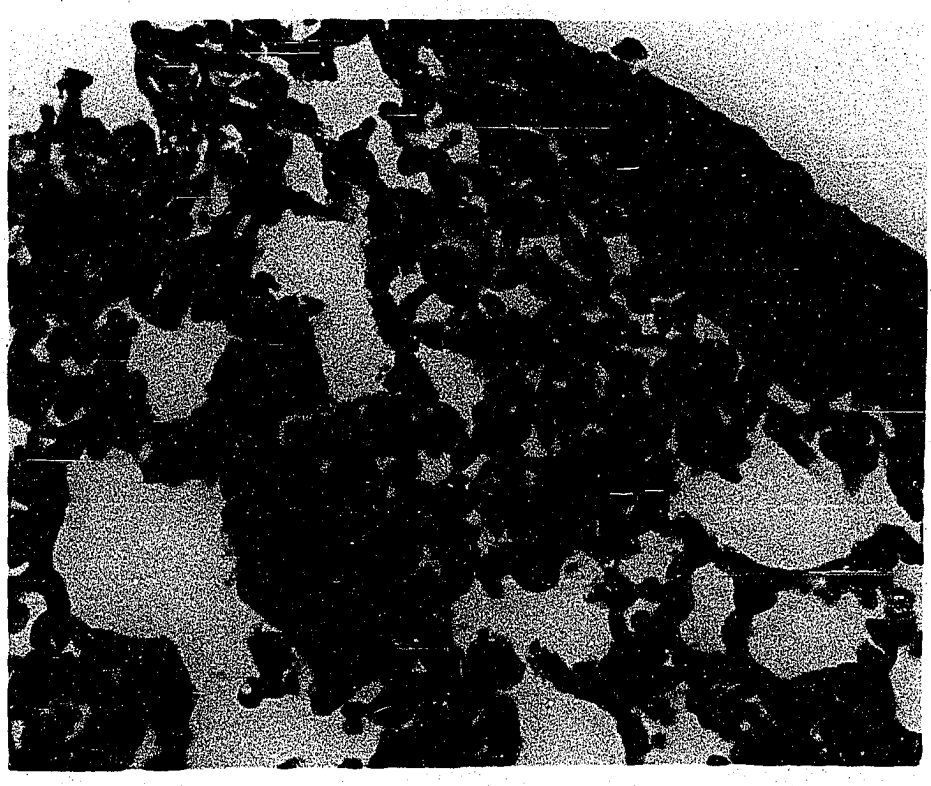
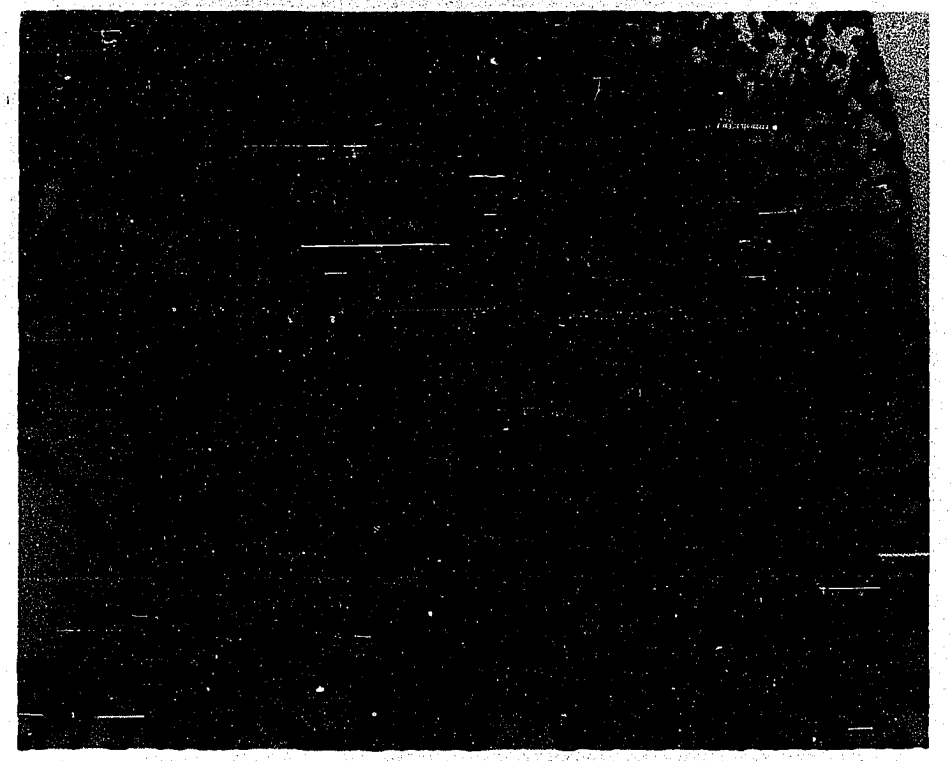


Figure 32. Mouse lung exposed to an aerosol of neodymium oxide for 30 days. Pneumonia is present. x 67. H. and E. stain.

Figure 33. Higher magnification of pneumonic area. Arrows point to macrophages laden with neodymium. The foreign material could be seen more clearly with diffuse lighting. Neutrophils are prominant. x 450. H. and E. stain.



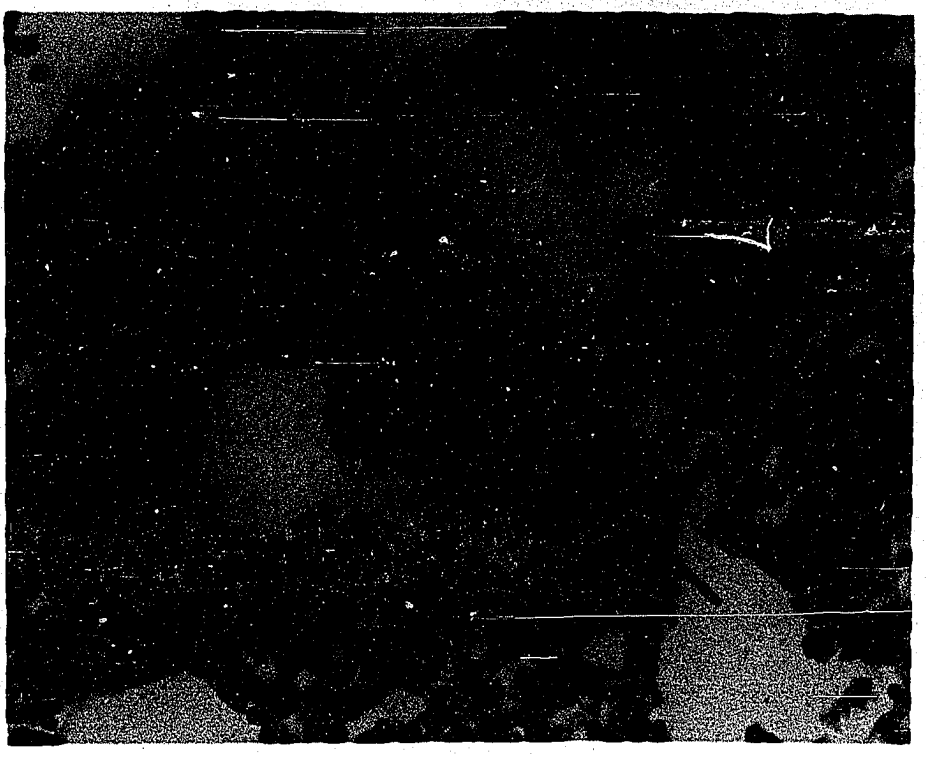
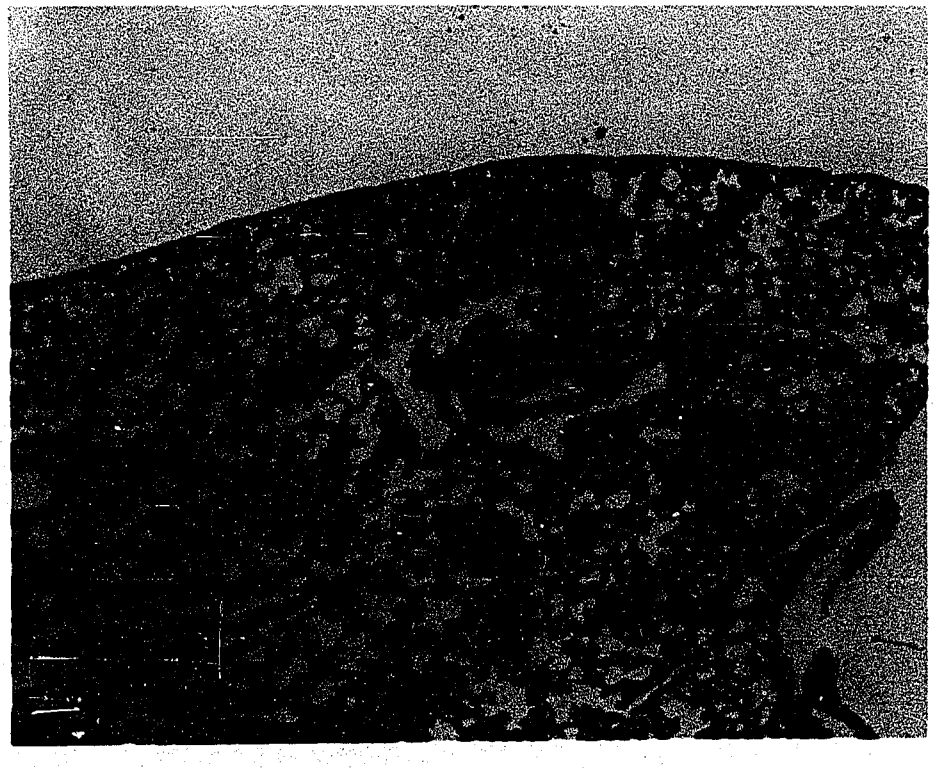
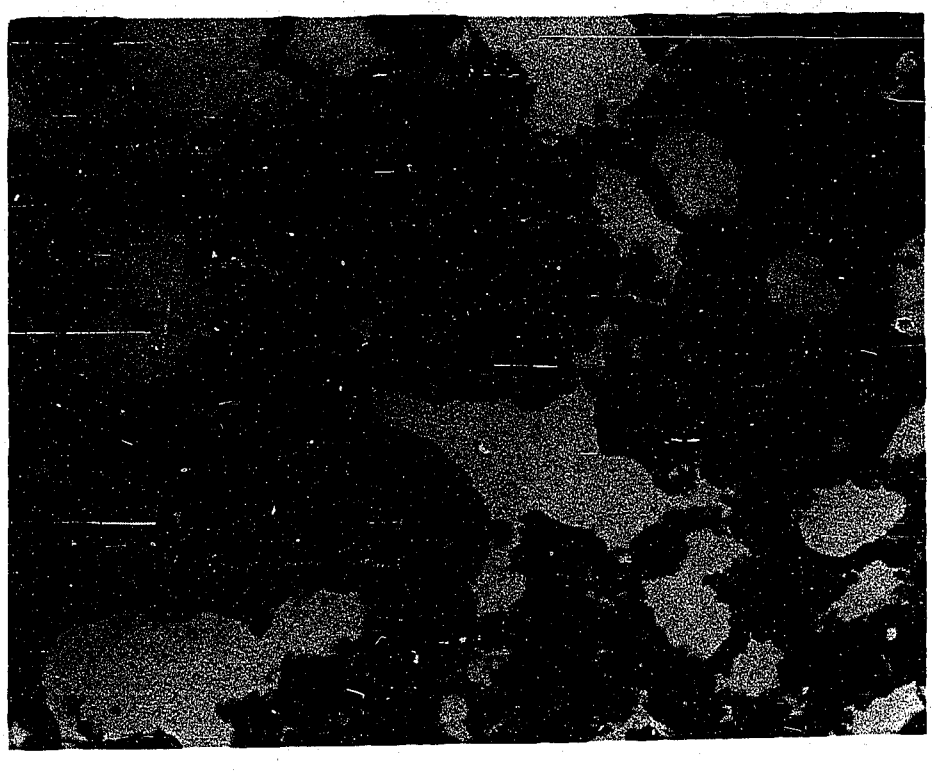


Figure 34. Lung from a mouse exposed to an aerosol of neodymium oxide for 70 days. x 67. H. and E. stain.

Figure 35. Higher magnification of lung seen in Figure 34. Macrophages heavily laden with neodymium are evident. x 450. H. and E. stain.





After 80 days additional focal pneumonia was seen and mild emphysema was present in less dependent portions of the lungs (Figures 36 and 37). At 100 days neodymium could be seen in macrophages in the tracheobronchial lymph nodes (Figure 38). By 110 days the lymphocytic response in most mice was extensive (Figure 39). At this time some lungs appeared to be 50 per cent involved.

At 120 days (Figures 40 and 41) the lungs presented a picture of severe impaction. The most striking finding, however, was the consistent lack of any dramatic tissue reaction to the foreign material. The lung parenchyma responded only by a swelling of the mural epithelium, the component cells having enlarged. No doubt these partly constituted the source of the cells which eventually came to occupy the alveolar spaces. These cells varied in appearance from large single and multiple nucleated foamy cells containing neodymium to more dense cells in which the nuclei could not be seen because of the concentration of neodymium in the cell (Figures 41 and 42). Neodymium was found in alveolar septal cells both free in the lumen and within the alveolar wall.

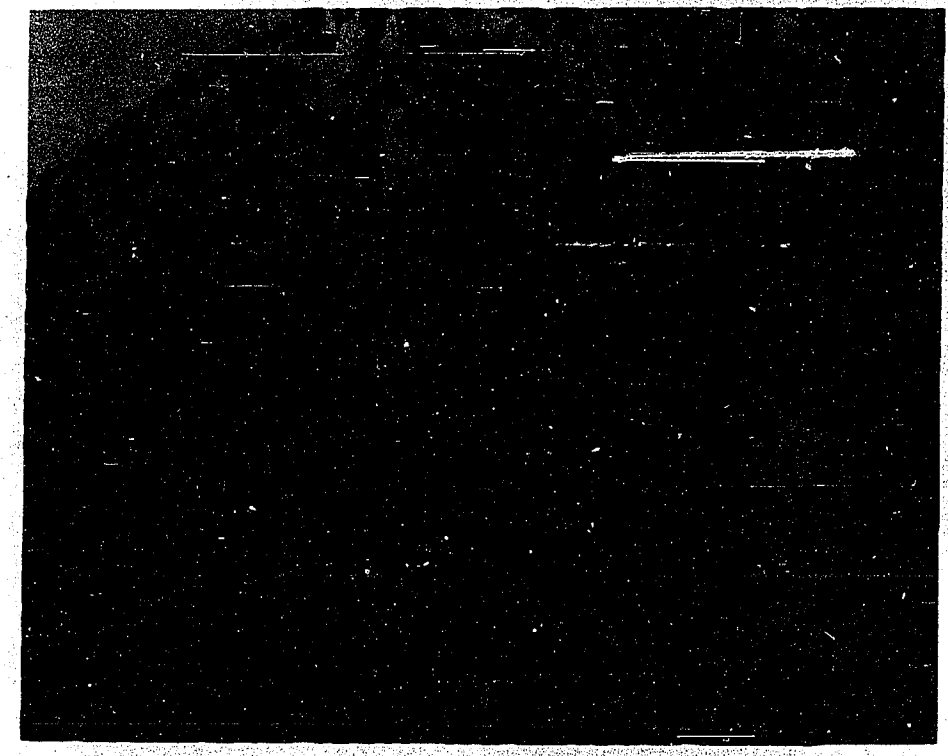
In one mouse exposed for 120 days an adenoma of a bronchiole was seen (Figures 44 and 45).

Staining with Gomori's one step trichrome method verified the absence of any excessive fibrosis in the lungs of these animals.

Normal direct lighting through the microscope tended to obscure the particles in the macrophages. Figure 43 represents a section taken with diffuse light which accentuates the particles. All photomicrographs were not taken this way since cellular detail was lost.

Figure 36. Lung from a mouse exposed to an aerosol of neodymium oxide for 80 days. Emphysema is present and lung parenchyma is extensively involved. x 67. H. and E. stain.

Figure 37. Higher magnification of lung seen in Figure 36. Arrow points to large rare earth laden macrophage. x 450. H. and E. stain.



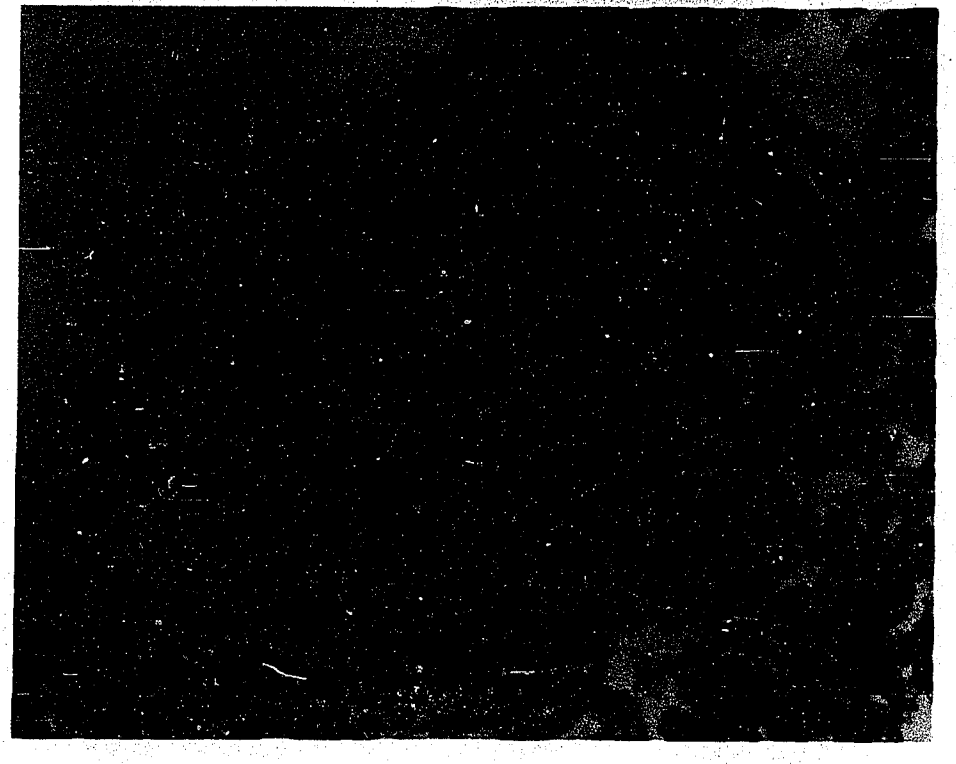
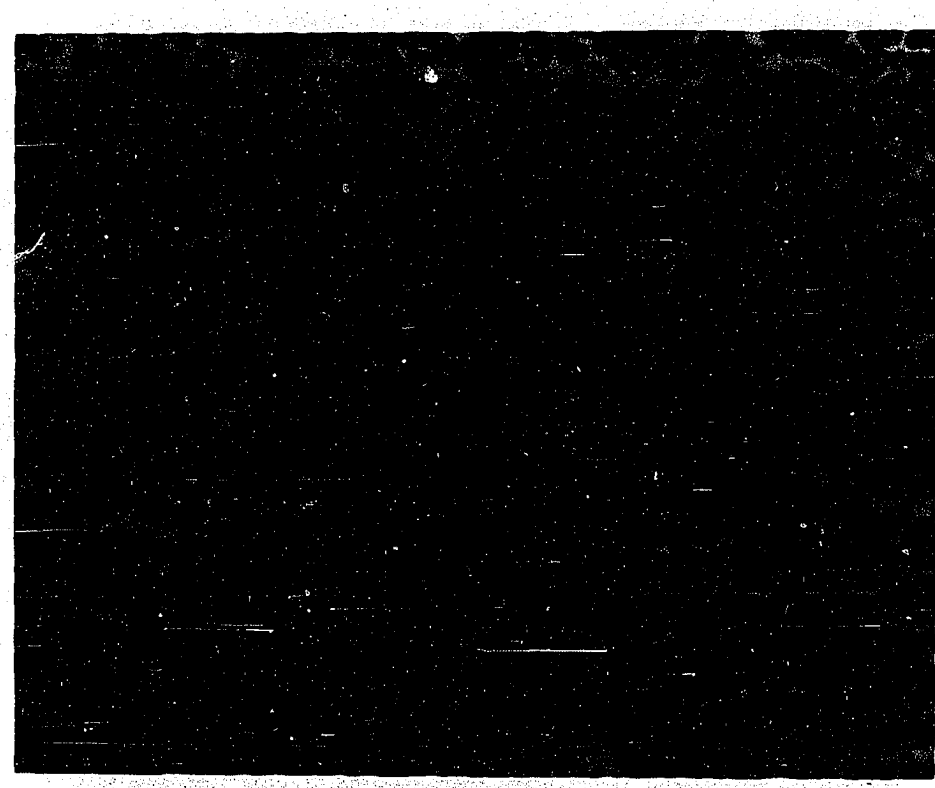


Figure 38. Tracheobronchial lymph node from mouse exposed to an aerosol of neodymium oxide for 100 days. Neodymium can be seen in macrophages. x 450. H. and E. stain.

Figure 39. Lung from a mouse exposed for 110 days to an aerosol of neodymium oxide. Arrows indicate areas of extensive peribronchiolar lymphocytic response. x 67. H. and E. stain.



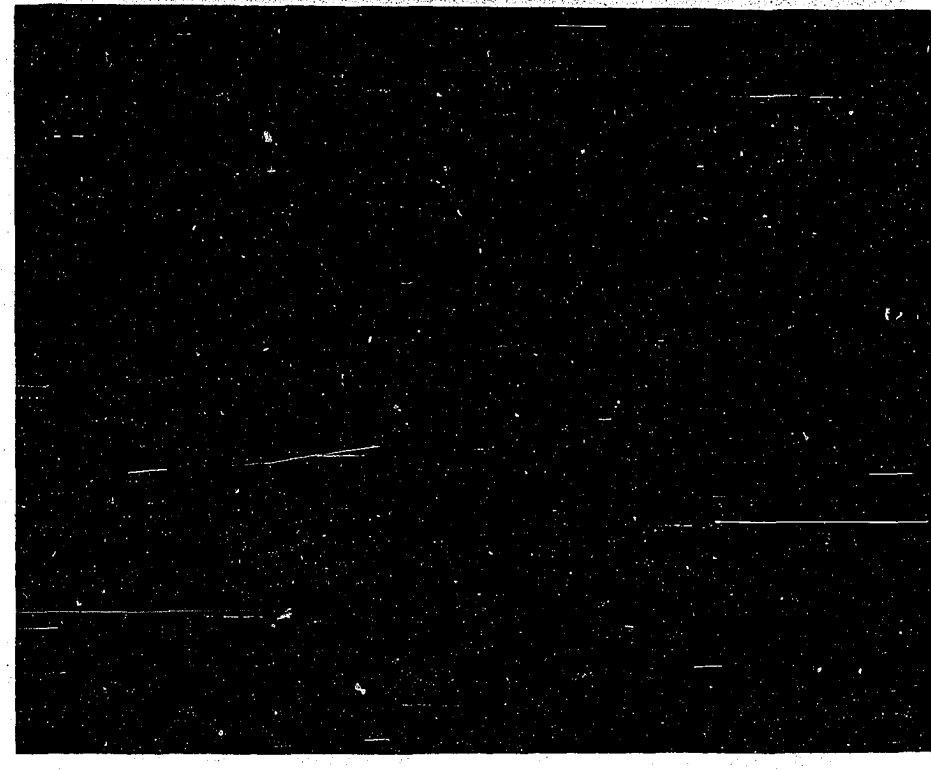


Figure 40. Lung from a mouse exposed to an aerosol of neodymium oxide for 120 days. Severe impaction is evident. x 67. H. and E. stain.

Figure 41. Higher magnification of lung shown in Figure 40. Many alveoli are filled with neodymium laden macrophages. x 450. H. and E. stain.

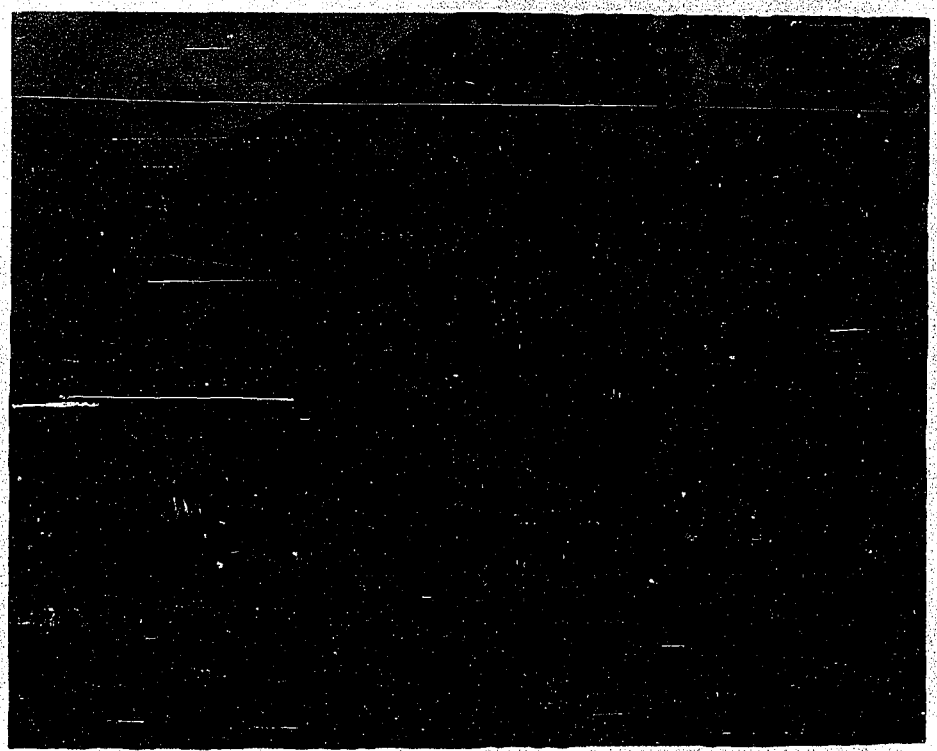
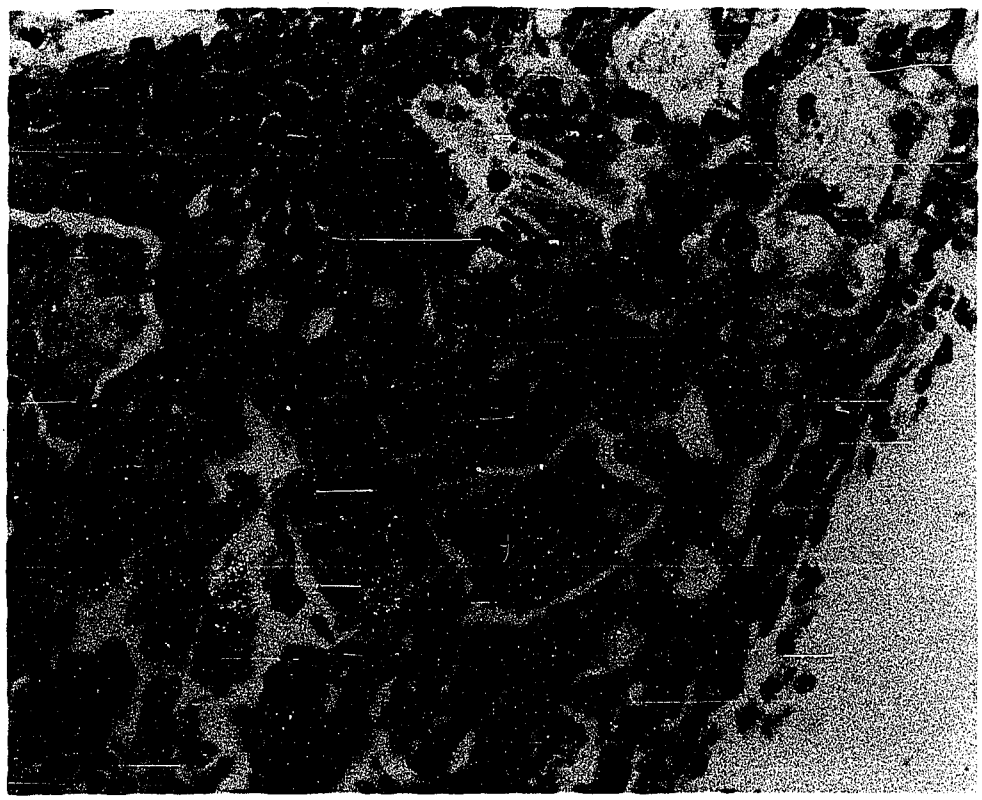


Figure 42. Multinucleated cells containing neodymium. Lung from mouse exposed for 110 days to an aerosol of neodymium oxide. x 450. H. and E. stain.

Figure 43. Lung from a mouse exposed for 60 days to an aerosol of neodymium oxide. This picture was taken with diffuse light to accentuate the particles in the macrophages. x 670. H. and E. stain.



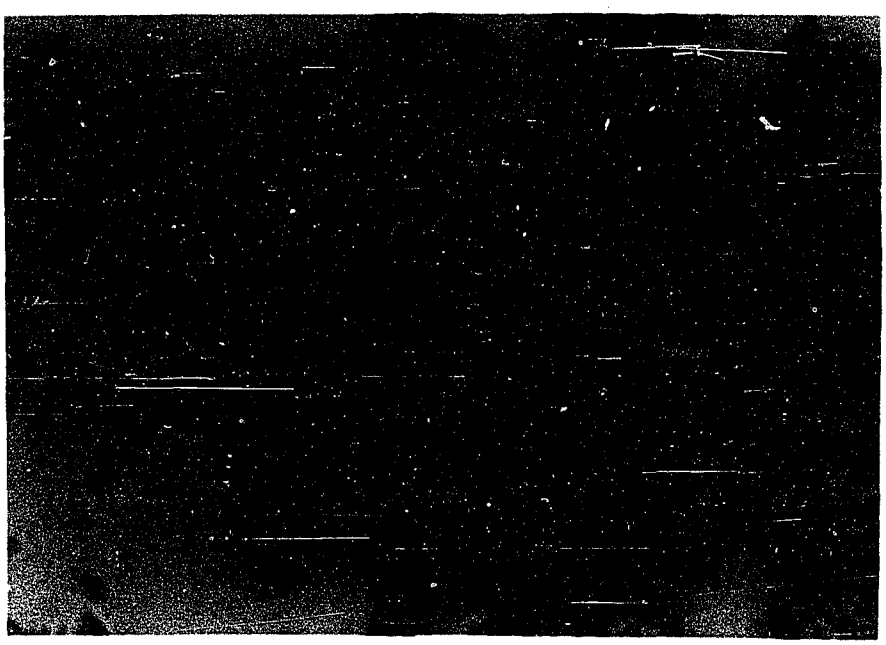
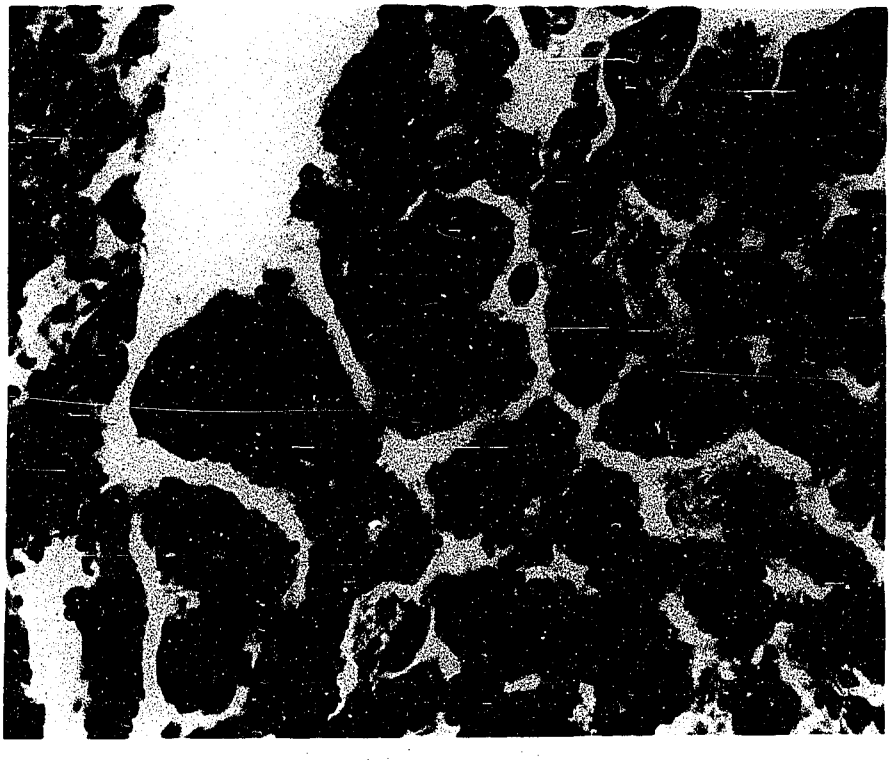


Figure 44. Adenoma found in mouse exposed to an aerosol of neodymium oxide for 120 days. x 67. H. and E. stain.

Figure 45. Higher magnification of the adenoma shown in Figure 44. x 450. H. and E. stain



There were no consistent microscopic lesions seen in any of the other organs or glands examined, and the lungs from control mice appeared normal (Figures 46 through 49).

Guinea pig lungs progressed through essentially the same series of changes as the mice (Figures 50 through 59), and by the end of 120 days the fundamental pattern of response by the lung tissue to the inhaled neodymium oxide was established. In the guinea pigs, however, the response was more dramatic. The dust appeared in the nodes at 50 days (Figures 60 and 61) and the lymphocytic hyperplasia was more striking. These changes combined to present a lung that appeared to be more severely involved than in the mice.

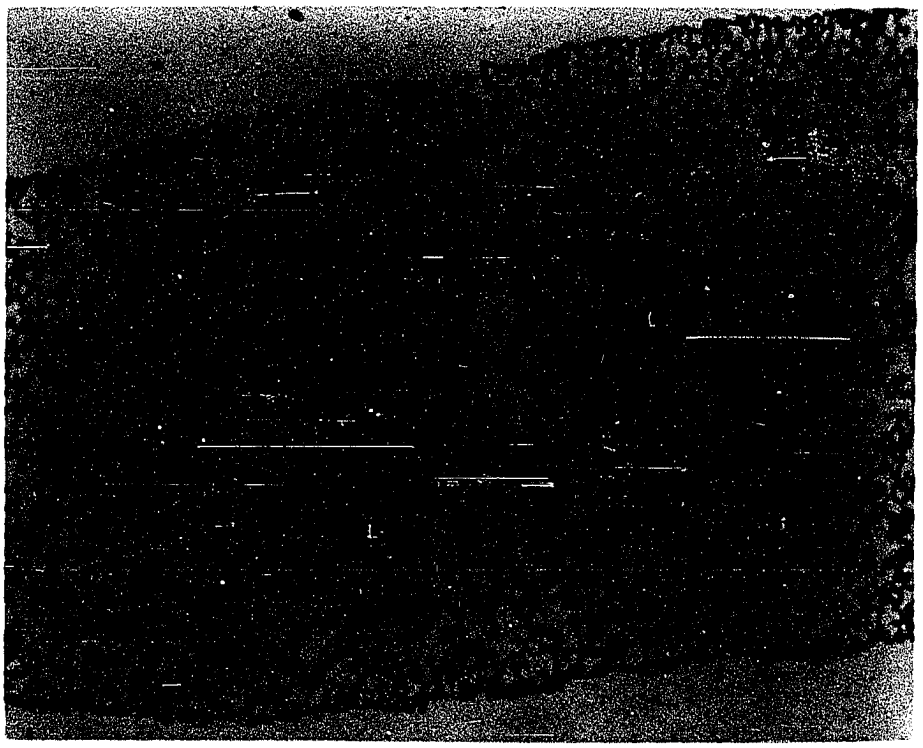
It is emphasized that in the guinea pig experiment the control lungs also showed some septal thickening, atelectasis and lymphocytic hyperplasia (Figures 62 and 63). This finding of a more dense parenchyma, particularly in the 10, 20, and 30 day animals, made the early response to dust look more severe than it probably was. Control lungs past the 30th day had a tendency to present less of this atelectatic appearance than those before (Figures 64 and 65).

In one guinea pig exposed for seventy days to neodymium oxide a chronic fibrosing pneumonia was observed (Figures 66 and 67). The significance of this finding is not known. It did display hyperplastic tendencies in the peribronchiolar areas. Some rare earth could be seen in the affected tissue.

Heart, liver, spleen, mesenteric lymph nodes, testis or ovary, kidney, adrenal, and bone marrow remained normal.

Figure 46. Lung from control mouse held in inhalation chamber but without neodymium oxide for 20 days. x 67. H. and E. stain.

Figure 47. Higher magnification of control lung shown in Figure 46. x 450. H. and E. stain.



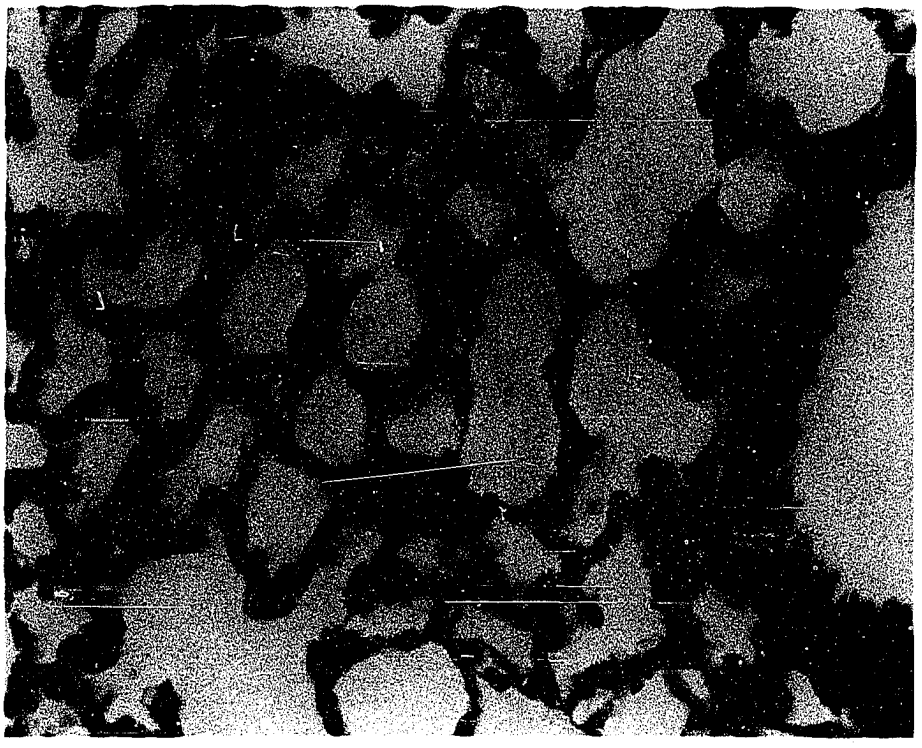


Figure 48. Lung from control mouse held in inhalation chamber but without neodymium oxide for 120 days. x 67. H. and E. stain.

Figure 49. Higher magnification of control lung shown in Figure 48. x 450. H. and E. stain.

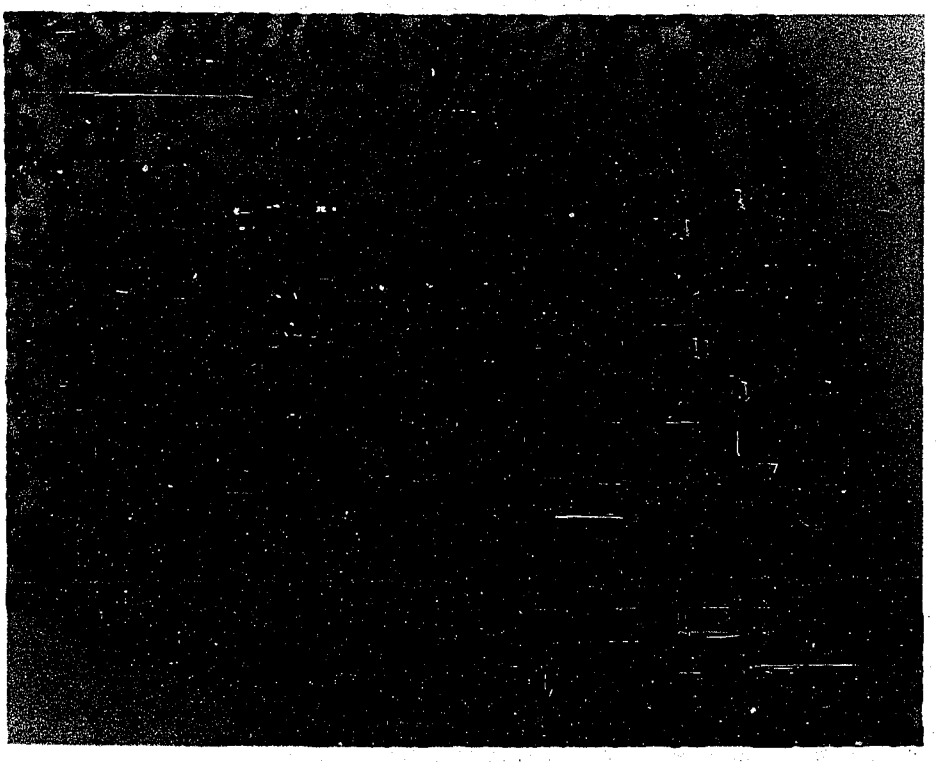


Figure 50. Lung from guinea pig exposed to an aerosol of neodymium oxide for 10 days. x 67. H. and E. stain.

Figure 51. Higher magnification of the same lung shown in Figure 50. Arrows point to typical macrophages in early response to foreign material. x 450. H. and E. stain.

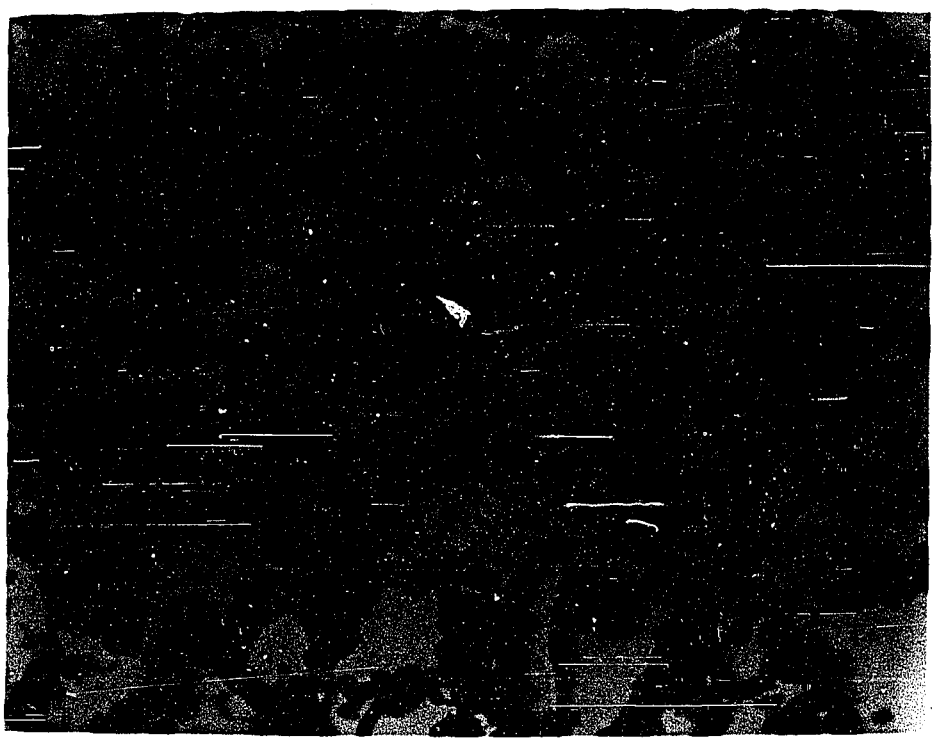


Figure 52. Lung from a guinea pig exposed to an aerosol of neodymium oxide for 40 days. Lymphoid response and atelectic appearance is not unusual in most guinea pigs lungs. x 67. H. and E. stain.

Figure 53. Higher magnification of lung shown in Figure 52. Arrows point to typical multinucleated cells containing neodymium. x 450. H. and E. stain.

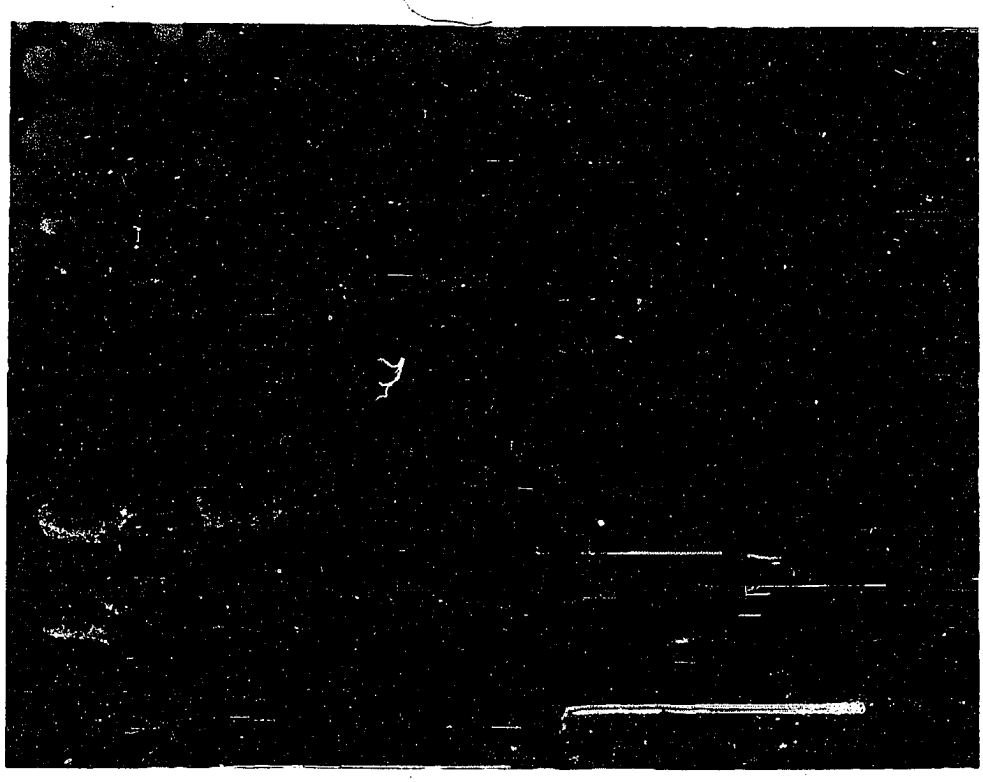
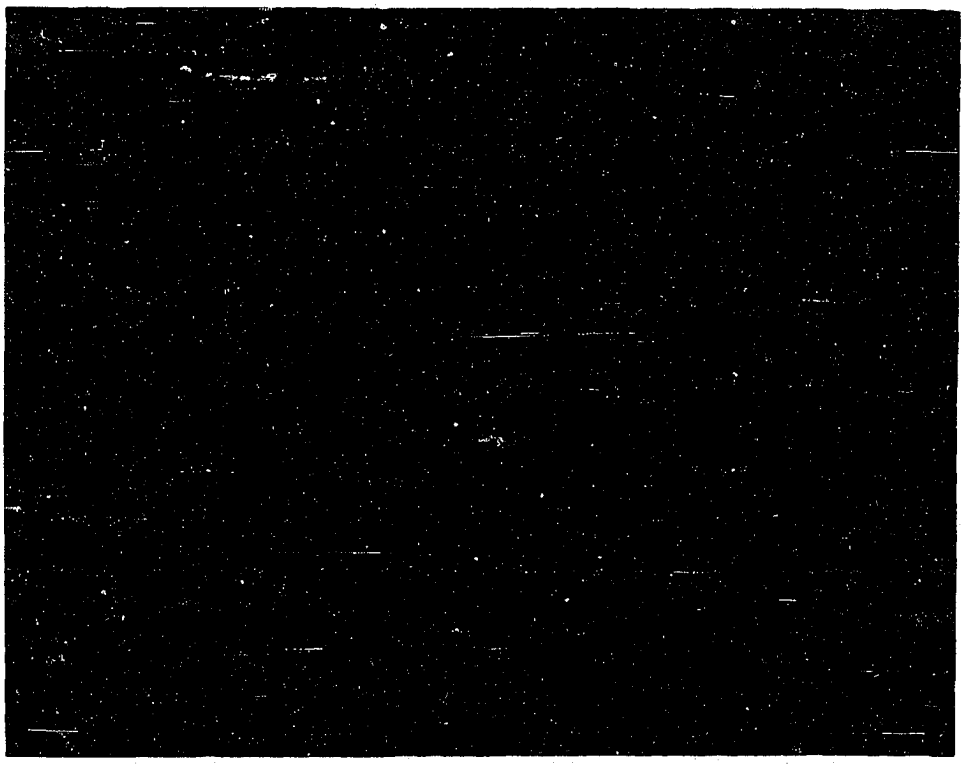


Figure 54. Lung from a guinea pig exposed to an aerosol of neodymium oxide for 60 days. x 67. H. and E. stain.

Figure 55. Higher magnification of lung shown in Figure 54. Arrow points to macrophage packed with neodymium. x 450. H. and E. stain.



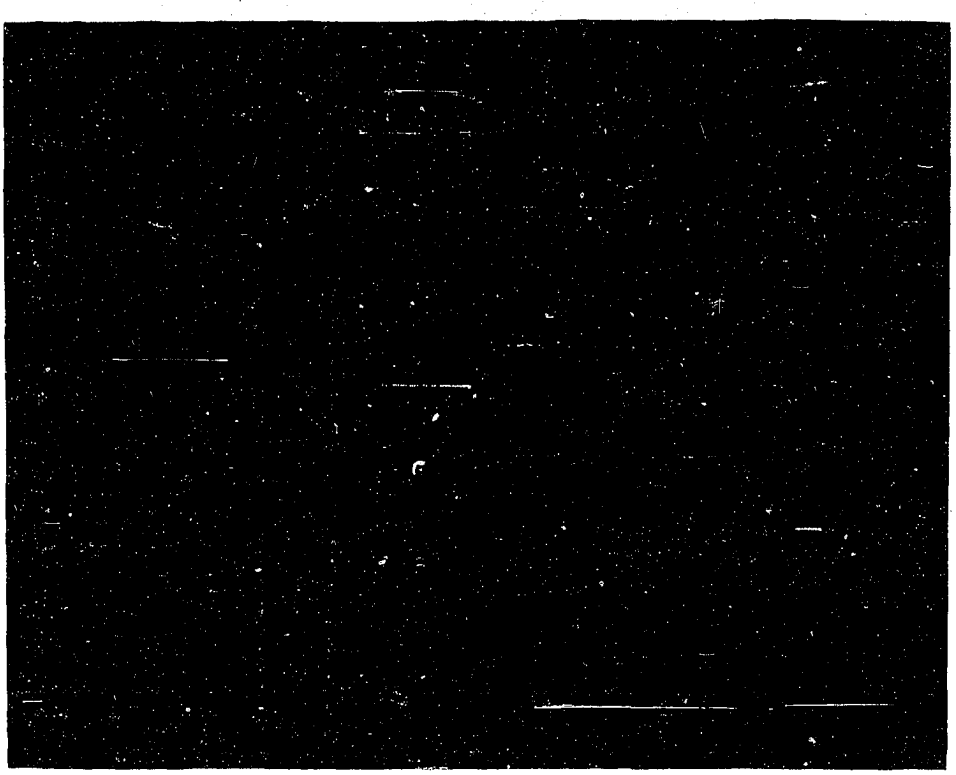


Figure 56. Lung from a guinea pig exposed to an aerosol of neodymium oxide for 110 days. Dark neodymium containing cells are visible, and impaction is severe. x 67. H. and E. stain.

Figure 57. Higher magnification of lung shown in Figure 56. Heavily laden macrophages are prominant. x 450. H. and E. stain.

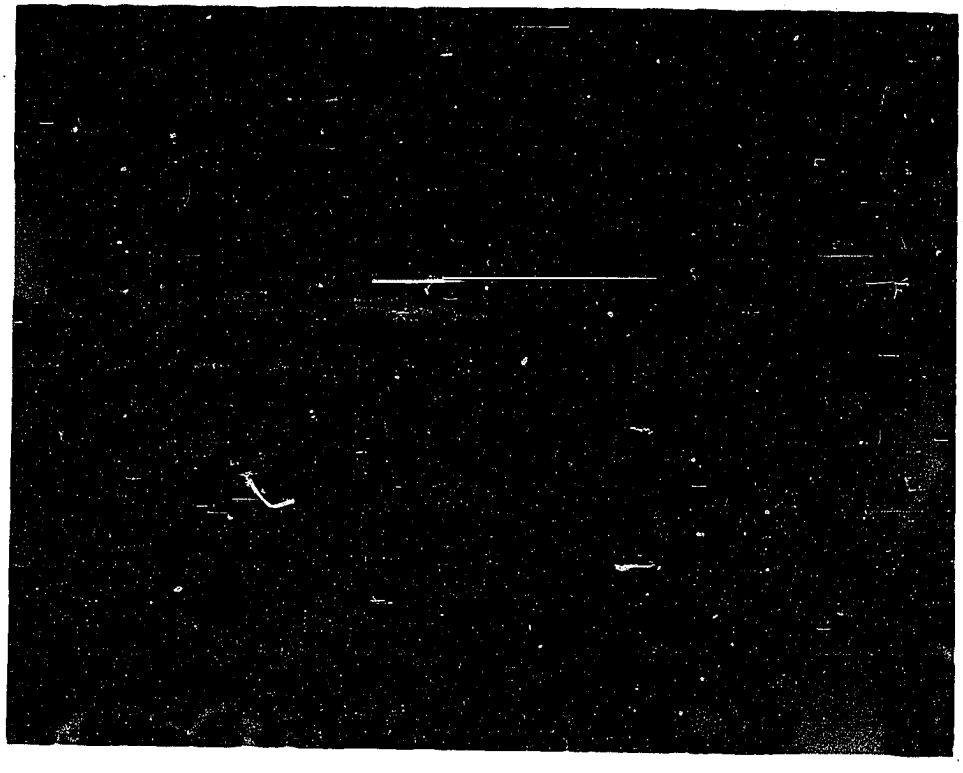
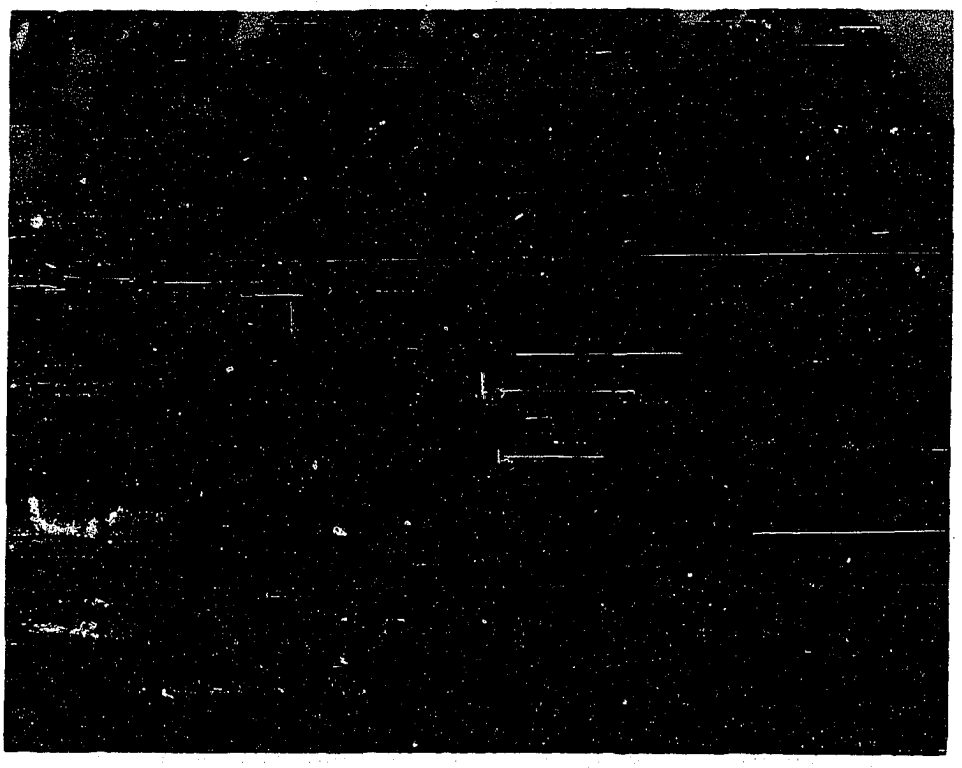


Figure 58. Lung from a guinea pig exposed to an aerosol of neodymium oxide for 120 days. x 67. H. and E. stain.

Figure 59. Higher magnification of the lung shown in Figure 58. Impaction is severe and macrophages packed with neodymium are prominant. x 450. H. and E. stain.



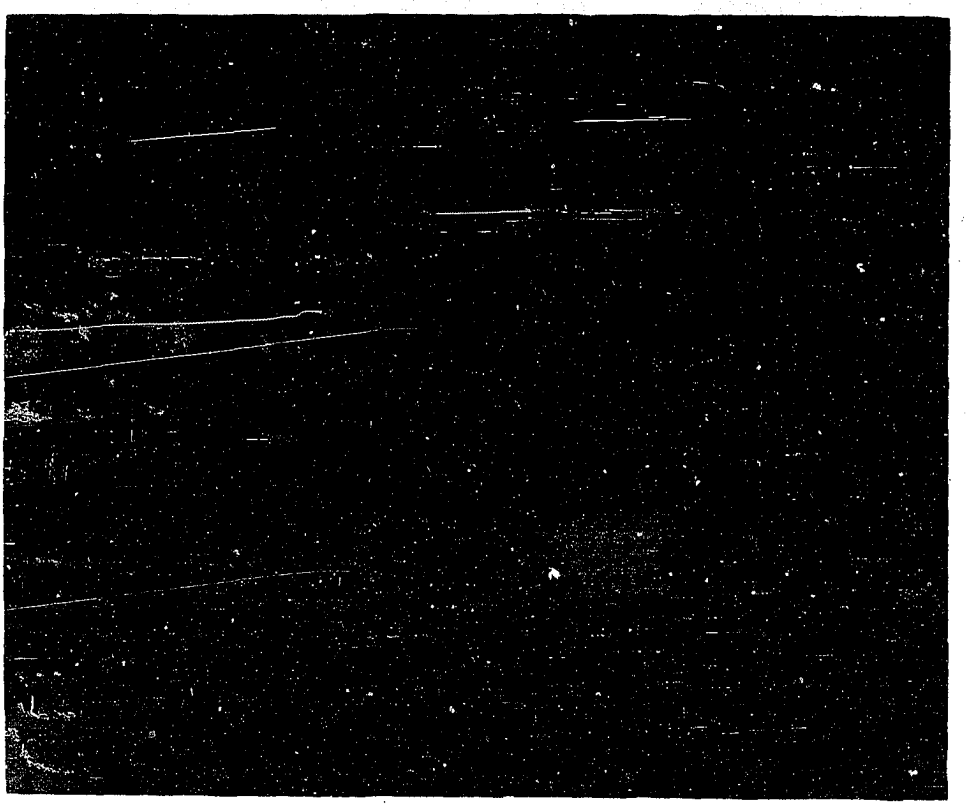
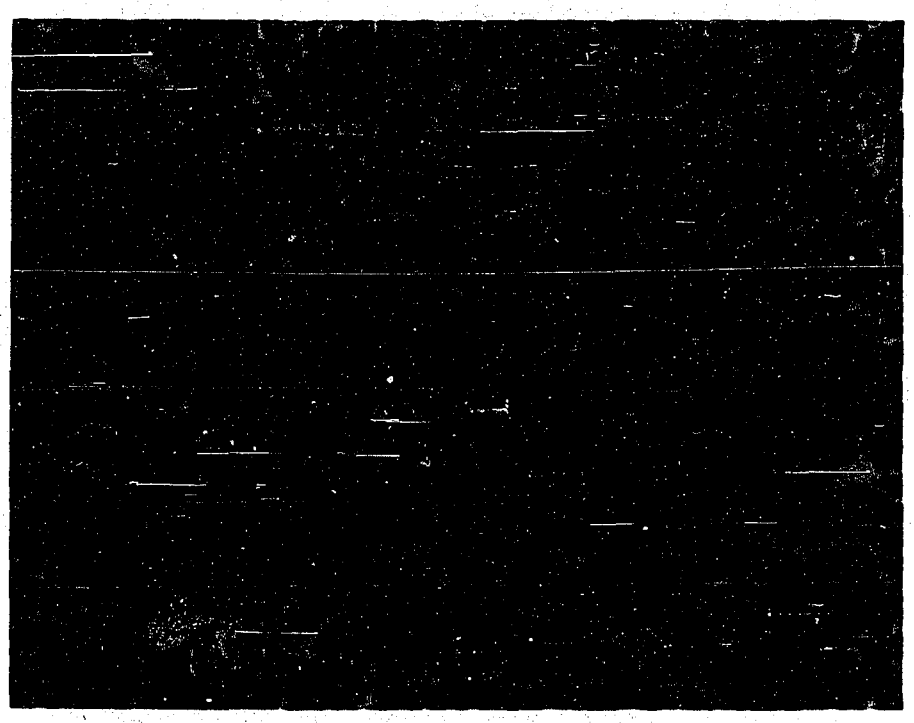


Figure 60. Tracheobronchial lymph node of a guinea pig exposed to an aerosol of neodymium oxide for 50 days. Arrows point to macrophages containing neodymium. x 450. H. and E. stain.

Figure 61. Tracheobronchial lymph node of a guinea pig exposed to an aerosol of neodymium oxide for 120 days. Arrows indicate a large nest of macrophages packed with neodymium. x 450. H. and E. stain.



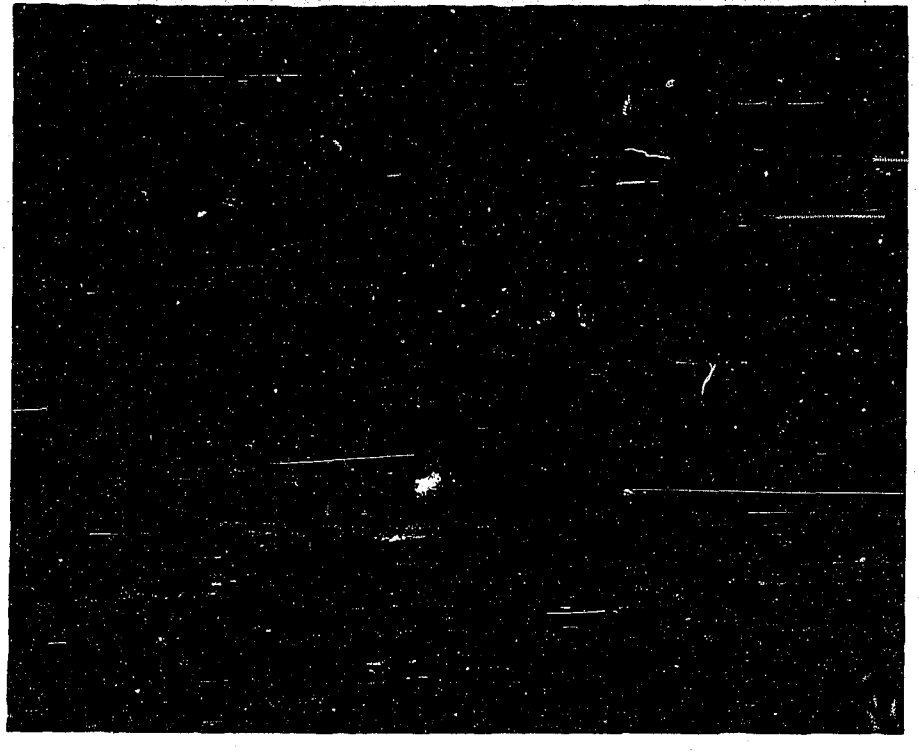
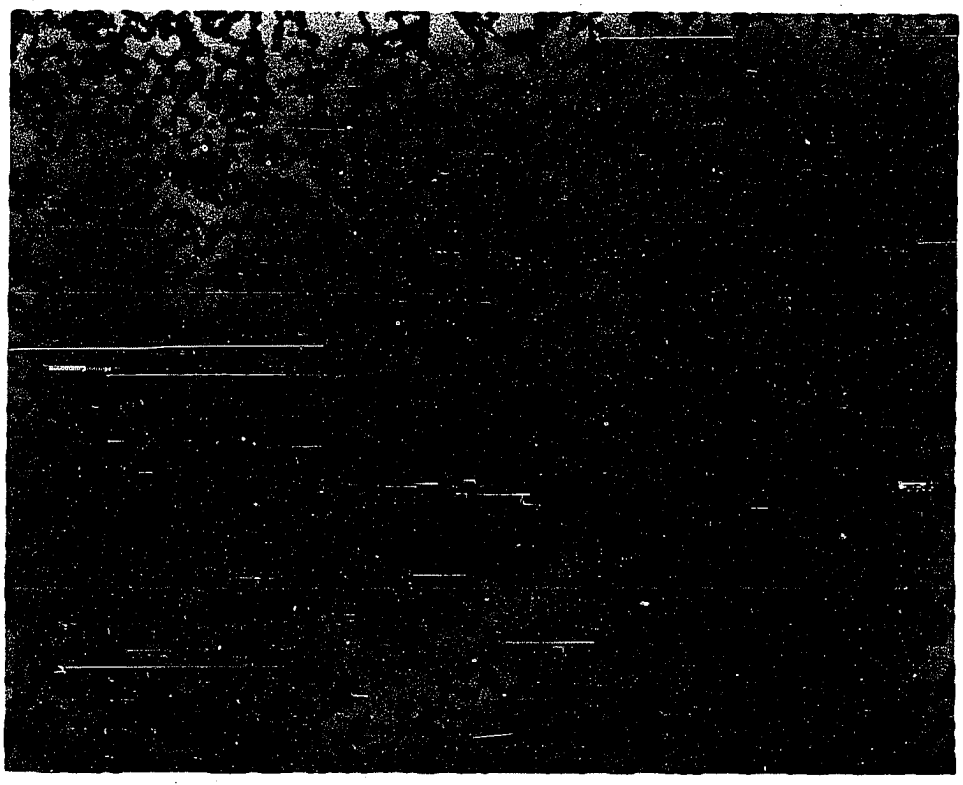


Figure 62. Lung from a control guinea pig held in an inhalation chamber through 20 exposure days. Thickened septa and lymphoid hyperplasia are present. x 67. H. and E. stain.

Figure 63. Lung from a control guinea pig held in an inhalation chamber through 30 exposure days. Dense atelectic appearance was common in control animals early in the experiment. x 67. H. and E. stain.

Figure 64. Lung from a control guinea pig held in an inhalation chamber through 100 exposure days. x 67. H. and E. stain.

Figure 65. Higher magnification of the lung shown in Figure 64. This is normal lung. x 450. H. and E. stain.



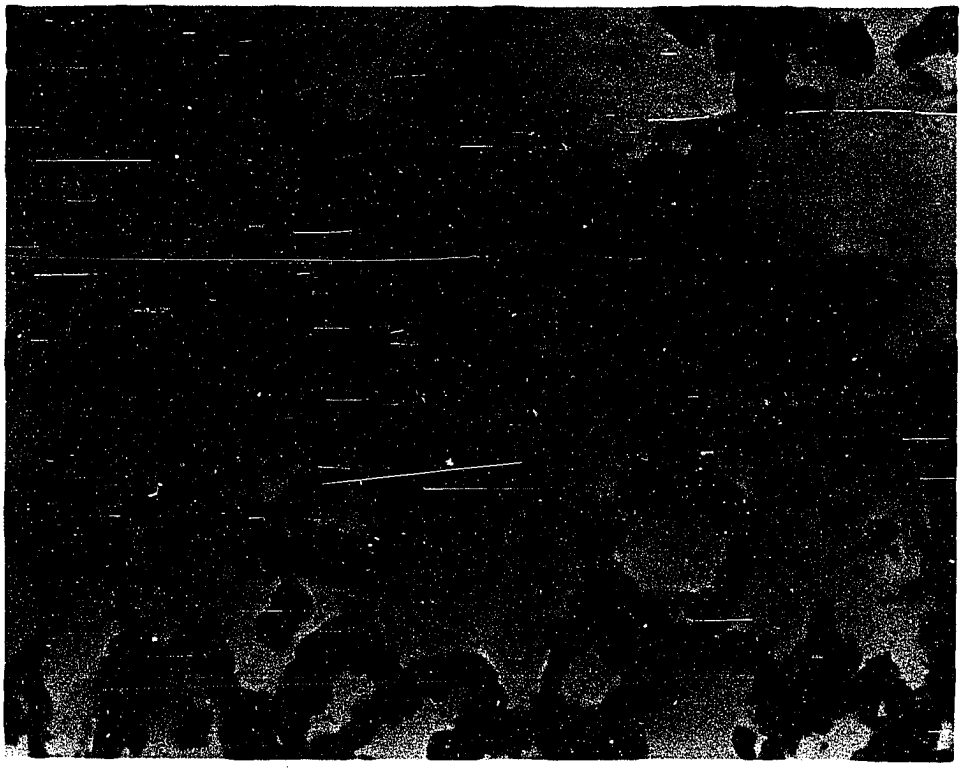
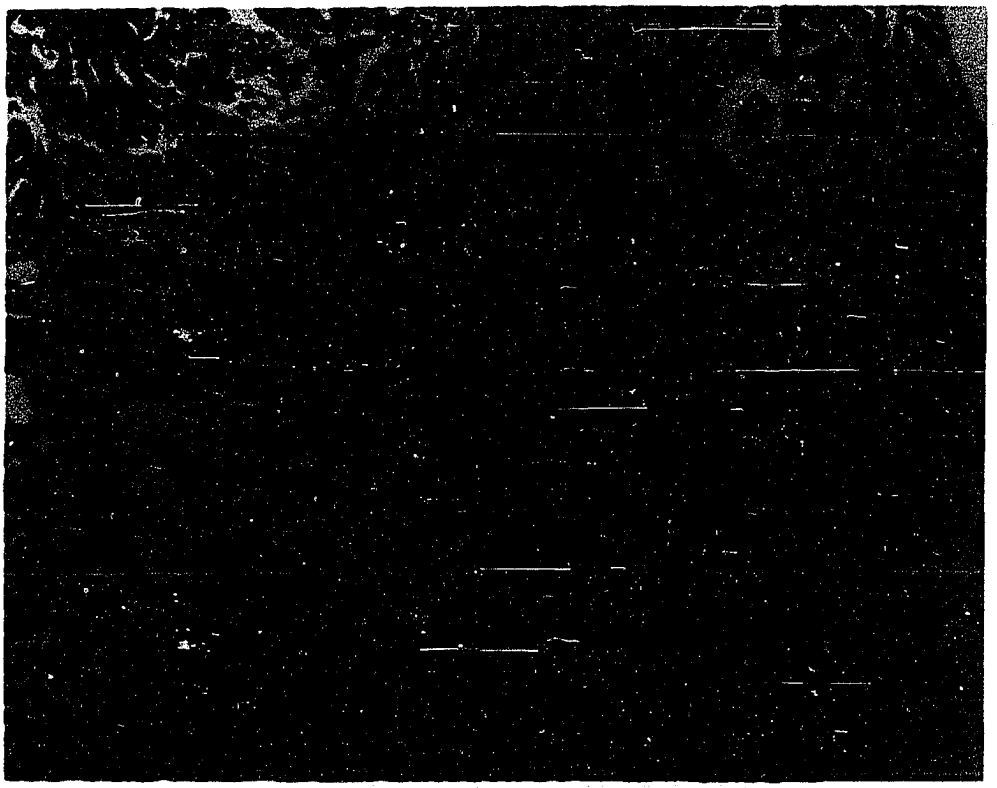


Figure 66. Chronic fibrosing pneumonia seen in one guinea pig exposed to an aerosol of neodymium oxide for 70 days. x 67. H. and E. stain.

Figure 67. Higher magnification of lung shown in Figure 66. x 450. H. and E. stain.



DISCUSSION

One of the most important parameters in any toxicity study is dosage. Normally this is an easily established value that can be stated in exact and precise terms. This is not so in inhalation toxicity studies. In this type of experiment a known amount of the agent being administered is not physically given. It can only be made available to the animal to be taken from the atmosphere during the normal process of breathing. Dosage is dependent on all of those factors which govern the availability and physical characteristics of the aerosol and the intake of air by the subject. Particle size of the aerosol is considered to be highly significant.

The basic mammalian respiratory apparatus is a system of various sized tubes and ducts ending in alveoli with tube diameters generally decreasing as the alveoli are approached. Air flow rates vary in these different sized tubes and produce direct physical factors opposing aerosol particle deposition in the alveoli. Larger particles will settle or be filtered out by hair in the nostrils. Others will adhere to the sticky mucous secretion from the ciliated respiratory epithelium. Smaller particles may be deposited more distally in the bronchial tree. Some of the dust will stay in suspension and be exhaled without having an opportunity for deposition. For these and other reasons deposition, retention, distribution, and clearance are all in part functions of particle size.

Morrow (1958) gave a brief but comprehensive review of the various physical forces involved in dust deposition in which he suggested that

the dominant forces were gravitational settling for the large particles (greater than 0.5 μ diameter) and Brownian motion impaction for the small particles (less than 0.1 μ diameter). Other factors listed were inertial impaction, coagulation or aggregation, condensation, electrical charge, thermal effects and dust concentrations.

He stated that gravitational settling was important since particles in the region of 20 μ diameter and of unit density fall with a terminal velocity of 10^4 μ/second. At 1 μ diameter, the velocity decreases to 35 μ/second while a .01 μ diameter particle falls at only .04 μ/second. Brownian motion was said to be important since particles below 0.1 μ diameter show an increasing susceptibility to random motion imparted by air molecules. These particles are characterized by a series of relatively high velocity excursions of short duration in random direction. In the lower respiratory tract this enhances deposition by propelling particles, which are too small to be moved by gravity, into the alveolar walls.

These two factors could lead one to conclude that between 0.1 μ and 0.5 μ diameter is a region of particle size which is least affected by combined Brownian motion and gravitational forces, and should constitute the most stable aerosol configuration. Morrow (1958) emphasized that there was a growing misconception that this represented a range or region of particle size of relatively less importance to deposition than any other. This is a misconception for several reasons. The work supporting it has been based on unit density studies. While the size of the particle is the most significant factor in sedimentation, the density is also extremely important. This is particularly the case in studies as this one which involved the oxide of a heavy metal.

A 0.5 μ diameter particle with a density of 10 has the sedimentation characteristics of a unit density particle of about 2 μ diameter. For this reason smaller particles of heavier aerosols contribute a larger percentage of the aerosol deposited. Conversely in calculating effects of Brownian motion, in accord with the Einstein equation, density is not involved. It seems logical that in high unit density aerosols such as neodymium oxide the stable area between 0.5 μ and 0.1 μ would be removed. This range would become very important to deposition. Morrow (1958) also concurred in regard to this with unit density aerosols and stated that, "If the significance of the exposure is based on the penetration and retention of relatively insoluble material in the parenchymatous areas of the lungs, the role of the large particles (greater than 1 μ) is probably of little consequence. Moreover, the relatively high deposition and retention of small particles (less than 1 μ) in the lung parenchyma has been repeatedly demonstrated."

Punte (1956) in a study of particle size effects using paraoxon in rats stated that it was generally accepted that the toxicity of an aerosol increased with a decrease in particle size and that the optimum particle size for maximum inhalation toxicity of particulate material was considered to be below 1 μ.

Stokinger (1949) concluded from data on the toxicity of inhaled uranium dioxide that the intensity of response was a function of particle size, the greater response being associated with smaller particles and vice versa. He reported that particles larger than 1.0 μ were less toxic than those smaller than 1.0 μ.

The aerosol to which mice and guinea pigs were exposed in this investigation was of an effective size range.

A whole-body exposure system was used in preference to a nose-only system for several reasons. Those systems in which only the animal's nose is in contact with the contaminated atmosphere require that the animal be either heavily tranquilized or anesthetized. The animal must be closely confined and exposure periods must be short. The first two factors constitute a deviation from normal or physiological breathing patterns and the last factor precludes exposures that would resemble low level industrial exposures for a six or eight hour working day.

It was believed that the additional exposure that animals received from licking their hair was not of great consideration in this work since the oxide used was of low solubility. Particulate material that is returned from the bronchial tree and swallowed would constitute an oral exposure regardless of whether a nose-only or whole-body system was used. For this reason neither system challenges only the lungs.

The concentration of 30 mg of neodymium oxide/meter^3 of air in the chambers was too small to be seen in suspension. Accumulation of a fine layer of the dust on the cages was visible after several hours. Its presence in the air did not appear to distress either the mice or guinea pigs.

Since animals had to be removed from the chambers each evening and returned to the colony room where they were housed, watered, and fed, there was the danger of human exposure to neodymium oxide. This danger was minimized by shutting off the Wright Dust Feed Mechanism several minutes before the chambers were opened. The air moving equipment was

allowed to continue running and the chambers were flushed out with clean air. Gloves and a dust mask were worn by the technician handling the animals. The cages were wiped with a damp sponge before they were removed from the chamber. Routine monitoring of the colony room and the chamber room was accomplished by using a portable vacuum pump and the same procedure with which the concentration in the chambers was evaluated. This precaution revealed no rare earth in either of these areas.

Effects of inhaled neodymium oxide in both the mouse and guinea pig were similar and differed only in the time of appearance of the rare earth in the tracheobronchial lymph nodes, the degree of response of the lymphoid tissue in the pulmonary parenchyma, the atelectasis present in control guinea pig lungs, and the pneumonia found in mice. Other findings included lower weight of the male guinea pigs when compared to their controls, the fibrosing pneumonia seen in one guinea pig lung, and the incidental finding of a tumor in one mouse lung.

Appearance of neodymium in the tracheobronchial lymph nodes of the guinea pigs was dramatic, and it was seen 50 days earlier than in the mice. It seemed logical to conclude that the lymphatics of the guinea pig were more efficient in moving the contaminant out of the lungs and into the nodes. The neodymium laden macrophages did not elicit any active response in the lymph nodes of either the mice or guinea pigs. They appeared in groups or nests of cells which were easily seen with a scanning objective of the microscope after fifty days of exposure in the guinea pigs and after one-hundred days in mice.

This rare earth was not found in any other lymphatic tissue, organs or glands.

The more extensive lymphoid hyperplasia observed in guinea pigs was anticipated since this tissue, in this species, is highly responsive to any stimulus (Jung, 1958).

The atelectic appearance of the lungs which was seen in some control guinea pigs appeared to be more severe in those animals which were taken off experiment and killed at 10, 20, and 30 days. Jung (1958) made the observation that these atelectic regions are considered to be normal and that they result from the contraction of bronchi which are encircled by muscle.

The obvious improvement in the appearance of the control guinea pig lungs could logically be attributed to the moving of the guinea pigs from a central colony where conditions were more crowded and less desirable as far as ventilation and temperature control were concerned to the inhalation facilities. Animals were housed under more favorable environmental conditions in the latter facility, and they lived in an extremely clean environment for six hours a day in the inhalation chambers. Even under these conditions an occasional control lung was seen in which atelectic areas and lymphocytic hyperplasia were prominant. There were no inflammatory reactions seen in conjunction with any of these areas in the control animals. Mouse control lungs remained clear.

The appearance of pneumonia in the mice exposed to neodymium oxide was biphasic. It was first observed at about twenty days then subsided and reappeared at about one hundred days. Cultures of these lungs revealed Escherichia coli. It was concluded that the initial wave of

pneumonia was the result of stress imposed by the inhalation of neodymium oxide to which the animals eventually adapted. The second increase in incidence was considered to be the result of purely physical factors. At this stage the lung burden of inhaled neodymium acted, if in no other way, as a mechanical hinderance to respiration. This second stress phase was thought to have resulted in the focal pneumonia observed.

The slower rate of gain observed in the male guinea pigs which were exposed to neodymium oxide can be related to the observation that these animals fought more and were more restless than the controls. A satisfactory explanation of this phenomenon was not made.

The chronic fibrosing pneumonia seen in one guinea pig was a finding of unknown significance. Particulate neodymium was present in macrophages in the affected area; however, it was not as concentrated in that area as in the rest of the lung parenchyma. It is possible that this process was established before exposure of the guinea pig was started.

It was significant that the other organs, glands and hematological parameters remained normal. Neodymium as the oxide, hydroxide or carbonate can be solubilized by chelation with many substances such as citrate, acetate, oxalate and amino acids which are normal to a cellular or tissue fluid environment. This process would provide a method of transporting the rare earth to other tissues. However, the absence of hepatic degeneration which would have reflected disturbances of lipid metabolism and the lack of prolonged prothrombin and coagulation times indicated that the neodymium was not transported by the blood at a level high enough to affect these mechanisms which are sensitive to the rare earths.

For this reason the lymphatics were viewed as being the only major route of removal of the neodymium oxide from the lung.

An incidental finding was an adenoma of a bronchiole in one mouse exposed for 120 days to neodymium oxide. This is of interest, but not necessarily significant. This type of tumor is common and spontaneous in some strains of mice. It should be emphasized, nevertheless, that this was the only one that had been found in this specific colony of mice.

The absence of a specific, inflammatory response by the parenchyma or the interstitial tissues of the lungs and lymph nodes to inhaled neodymium oxide combined with the lack of effects on other parameters observed suggest that this material is relatively inert and non-toxic to the mouse and guinea pigs when inhaled.

This might be an unwise assumption for several reasons. Inhaled neodymium oxide elicited no active response by the body which was designed to remove it quickly. Neither was it separated or isolated from other tissues by the usual body defense mechanism, and it is emphasized that the expected granulomatous tissue reaction to a foreign body was not present. The absence of reaction and hematologic change would make knowledge of antemortem exposure difficult. This could be a major hazard since exposure can be insidious. Another major problem in industrial medicine lies in the fact that some metals, such as beryllium, have a long period of latency before they produce an effect. If this is the case with neodymium, lesions may not manifest themselves for months or even years. It must also be strongly emphasized that some toxic agents, again using beryllium as an example, which are harmful to the human lung have not produced lesions in the guinea pig or the mouse.

Experimental investigations in this thesis answered only a few of the obvious questions concerning neodymium oxide. Discussion in the preceding paragraphs alluded to the fact that these limited observations could be misinterpreted. For this reason more information must be made available before the toxic potential of neodymium oxide to the pulmonary system can be completely understood.

It was realized at the conception of this investigation, that the results could not be regarded as those of a true chronic study even though exposure was for 120 days. Because of this, the experiment was designed to have a chronic phase as well as the one in this thesis.

At the end of each ten day period twenty-four mice and twenty-four guinea pigs that had been exposed and eight mice and eight guinea pig control animals were taken off test in addition to those in this acute or 120 day experiment. These "chronic" animals are being held in a colony for life term evaluation.

All the guinea pigs are bled from the heart and 25 per cent of the mice are picked at random and bled from the tail at twelve and eighteen months. The same hematologic examination is made as was described in the "acute" experiment.

These animals are weighed monthly and records are kept for individual guinea pigs. Mouse weights are recorded as mean weights of cage populations.

As the chronic animals die a necropsy examination is performed, and the same tissues are saved for microscopic study as in the acute experiment.

The chronic study in which the exposure phase is already completed awaits only the death of the animals involved to be terminated. This period will be approximately three to five years in the guinea pigs and thirty to thirty-six months in the mice.

The acute and chronic phases of the inhalation investigation of this one rare earth oxide, neodymium, is only a portion of a larger investigation of the lanthanons as a group. An investigation of the oxide of gadolinium has just been completed using the same protocol as that used with neodymium except that animals were removed from test every twenty days instead of every ten days. The same acute and chronic phases of the investigation are being conducted. The results of the acute experiment have not yet been evaluated.

Yttrium oxide is being used in a long term study of its effects on the dog and monkey. In this work exposure is for six hours per day, five days per week to an aerosol concentration of 15 mg of yttrium oxide/meter^3 of air. This exposure is late in its second year and should be continued for a minimum of five years. Dogs and monkeys are given physical examinations including hematologic studies and X-ray plates of the respiratory system every six months.

The inhalation toxicity investigations mentioned here also lie in the framework of a larger program in which the lanthanons are being evaluated when they are ingested and when the pure metal is implanted subcutaneously.

Many more questions need to be answered. For example, the distribution studies done in the intratracheal pilot experiment should be expanded. They did not distinquish between the amount of rare earth

found in the blood in each organ and that incorporated in the other cellular elements and fluids.

Excretion studies should be carried out. At present, there is no data to indicate how the body rids itself of the stable element, if it does at all, after it is transported from the lungs.

The complete process should be repeated using a more soluble form of the rare earths. This could be accomplished by chelating these elements with other compounds of varying stability constants. All of these studies should aid greatly in our understanding of this interesting group of metals which has so recently been made available in a pure form.

SUMMARY

This experiment was designed to evaluate the effects of a dust aerosol of stable neodymium oxide of a known particle size range and concentration on the lungs, other major organs, blood and body weight of mice and guinea pigs when administered over various periods of time up to 120 days.

These objectives were accomplished in the following manner.

1. An inhalation chamber system was developed in which a uniform distribution of pure neodymium oxide could be maintained in the air for six hours a day.

2. The aerosol that was suspended in the chamber air was standardized and characterized. Grinding procedures were developed which yielded a dust in which sixty per cent of the mass was made up of particles less than one micron in diameter. The relationship of mass to size was established by direct counts from electron photomicrographs and by the use of a cascade impactor.

Chamber air was sampled daily and the concentration of neodymium oxide was determined by spectrophotometric methods.

3. A pilot experiment was completed in which neodymium as the oxide and the citrate chelate was injected into the lungs of rats and guinea pigs. Selected organs and glands were analyzed at 60, 90, and 120 days to determine distribution patterns and lung burdens at the end of 120 days.

4. Based on the pilot experiment a final experimental design was developed in which mice and guinea pigs were exposed to an aerosol

concentration of 30 mg neodymium oxide/meter^3 of air for periods of six hours per day five days per week and in which animals were taken off test after every ten exposure days. The longest cummulative exposures were for 120 days.

The following parameters were compared between exposed animals and controls.

(1) hemoglobin

(2) packed cell volume

(3) white blood cell enumeration

(4) white blood cell differential count

(5) coagulation time

(6) prothrombin time

(7) red blood cell enumeration

(8) weight gains

(9) necropsy examination

(10) histopathological examination of the lungs, tracheobronchial lymph nodes, heart, bone marrow, spleen, liver, adrenal, kidney, mesenteric lymph node and testis or ovary.

5. There were differences from control animals in the weight gains of the male guinea pigs. Those exposed did not gain as rapidly as the controls. It was speculated that this resulted from the fact that exposed males were more restless and fought more than the control males. The relationship of this restlessness to the exposure was not determined.

6. Gross and histological changes revealed a progressive impaction of neodymium in the lungs. It was contained primarily in macrophages (alveolar septal cells) and some neodymium was transported to the tracheobronchial lymph nodes where it could be seen after one-hundred days in

the mice and fifty days in the guinea pigs.

Lymphocytic hyperplasia was common but was not considered to be a specific response to neodymium.

Mechanical disruption of the normal architecture of the pulmonary parenchyma was usual after sixty days of exposure. This resulted from the heavy deposition of the foreign material.

There were no specific inflammatory reactions to the foreign material during the 120 day period of exposure and observation. The usual granulomatous reaction that is commonly associated with such foreign materials was not present.

An adenoma of a bronchiole in one mouse exposed for 120 days and a chronic fibrosing pneumonia seen in one guinea pig exposed seventy days were considered as findings of unknown significance.

Other organs remained relatively normal.

7. Even though the lung tissue and the lymph nodes appeared to tolerate neodymium for 120 days this does not predict what the tissue reaction would be after a period of one or more years.

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