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Effects of exercise and inhaled yttrium oxide on blood lactic acid, erythrocyte volume, and lung histology in dogs

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Physiology

EFFECTS OF EXERCISE AND INHALED YTTRIUM OXIDE ON BLOOD

LACTIC ACID, ERYTHROCYTE VOLUME, AND LUNG HISTOLOGY IN DOGS

by

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A Dissertation Submitted to the

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Approved:

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Ames, Iowa

1965

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INTRODUCTION

The term "rare earths" has its origin with the early discovery of these elements. The word "rare" arises from the fact that these elements were discovered in scarce minerals. The word "earth" comes from the facts that they were first isolated from their ores in the chemical form of oxides and that the old chemical terminology for oxide is earth. The words "lanthanides" and "lanthanons", which are derived from lanthanum, the first member of this series of elements, are also commonly used to describe the rare earths.

Specifically, the rare earth elements (the lanthanons) include those of atomic numbers 57 through 71. They are members of the Group III A elements and appear in the sixth row of the Periodic Table. Yttrium, atomic number 39, is also a member of the Group III A elements. Because it is always found associated with the rare earths in ores and resembles them in chemical properties, yttrium is very often included by common consent. Yttrium was discovered by Gadolin in 1794 and obtained in a more pure form by Mosander in 1843.

The interest of the Atomic Energy Commission in the rare earth elements has resulted in methods being devised whereby the individual members of the series can now be obtained in high purity, and a number of companies have adopted these methods or modifications of them. As a result, rare earths can be obtained commercially in any desired quantity at

reasonable prices.

The more recent availability has led to numerous industrial uses. At present the rare earths are used as catalysts, paint driers, glass decolorizers, ceramic opacifiers, activators for fluorescent lighting, and reagent chemicals. They are valuable, also, in photosensitive glass, electronic equipment (such as vacuum tubes, capacitors, lasers, masers, and ferrites), sunglasses, and welders' goggles. The steel, iron, copper, aluminum, and magnesium in automobiles and other metal products contain small amounts of rare earths to improve their properties.

In addition to their industrial uses, the lanthanons and yttrium are also used in medical therapy. In the past they have been used as antiseptics, anti-motion sickness drugs, and as anti-tuberculosis agents. Today they are being used therapeutically as anti-thrombotic agents and in the treatment of certain tumors.

Rare earth metals and yttrium are also of interest because of their occurrence among fission products. Radioactive fission products are formed both in reactors and in nuclear weapons. At least ten radiolanthanons and six radioisotopes of yttrium are formed.

Because of their increased use in industry and medicine and because of their occurrence as fission products, the Division of Biology and Medicine of the United States Atomic

Energy Commission has been very active in the investigation
of lanthanon and yttrium toxicity. The toxicity of the stable
elements is of interest not only from the standpoint of their
use in industry and medicine but also as a subtraction factor
for assessment of their radioactive isotope toxicity.

In past experiments, the evaluation of the toxicity of
inhaled, stable rare earth oxides has been accomplished by
chemical, histopathologic, radiologic, and hematologic inves-
tigation. It is of interest to determine their effect upon
an individual's ability to do work. Impaired work performance
could be the end result of an otherwise obscure or subtle
toxicity.

Previous experiments dealing with the inhalation of stable
rare earths and yttrium pointed out certain histological
changes in the lung parenchyma such as alveolar wall thicken-
ing and extensive macrophage formation. No indication was
given in regard to impairment of function in such an exposed
individual. If sufficient alveolar wall thickening occurred
and if impaction of rare earth and yttrium in the lung con-
tinued with increased exposure, then a point may be reached
where there is interference with diffusibility of oxygen.
This might not be observed unless a stress was imposed over
and above the normal compensatory mechanisms of the body.
Therefore, by requiring treadmill exercise of exposed sub-
jects it could be assumed that altered diffusibility of oxy-
gen would be reflected in increased levels of blood lactate

by virtue of an increase in anaerobic glycolysis. The systemic effects of inhaled materials could also cause subtle damage to hematopoietic organs and cellular enzyme systems which also might be reflected in increased blood lactate levels during exercise.

This research primarily evaluates the effect of a dust aerosol of stable yttrium oxide on blood lactic acid of dogs subjected to treadmill exercise. It was assumed that the primary site of damage would occur at the level of the alveolar membrane and be reflected in a lower diffusibility of oxygen. Changes in lung histology were studied to correlate with this premise. In order to detect systemic effects upon hematopoietic organs, red cell volume, plasma volume, hemoglobin and white cell counts were also determined.

In addition to serving for the toxicologic evaluation of yttrium, it is supposed that this method of evaluation might be useful as a research technique for the evaluation of drug and food additive toxicity.

REVIEW OF LITERATURE

Toxicity, Distribution and Excretion
of Yttrium and Certain Rare Earths

The rare earths, atomic numbers 57 through 71, are members of the Group III A elements and appear in the sixth period of the Periodic Chart of the Atoms. Yttrium, atomic number 39, is also a member of the Group III A elements but appears in the fifth period. Although yttrium is not actually a rare earth, it is commonly classed with them because it is always found associated with the rare earths in ores and closely resembles them in properties (Spedding, 1951; Moeller, 1955; Gschneidner, 1964).

The chemistry of the rare earth metals is reviewed in a number of publications. Examples of these are the surveys of Vickery (1953) and Moeller (1963). The significance of the rare earths in biochemical and medical research has been illuminated by Kyker and Andersson (1956). A summary of the whole body of lanthanons has been given by Kyker (1962).

The importance of knowing the toxicity of yttrium and the lanthanons becomes apparent when one considers their use in industry and medicine and their occurrence among fission products following the detonation of nuclear weapons. Industrial production and use of yttrium and the lanthanons bring with them biological problems of a hygienic nature. In working with them, the air can contain air-borne metal particles

which can be inhaled. This has been emphasized by Magnusson (1963) and Kyker (1962). For irradiation of tumors, the question of suitable radioisotopes arises. One third of the radioisotopes which are used for medical therapy are lanthanons (Brucer, 1952). Due to its half-life (61 hours) and radiation energy (E_{max} 2.24 MeV), yttrium-90 has been the foremost one to come into use for this purpose (Magnusson, 1963). Siegal et al. (1956) report on the value of palliative treatment in man with yttrium-90. Their patients had cancer with pleural and peritoneal effusions. A comparison between yttrium-90 colloid and gold-198 colloid after intravenous administration shows that yttrium-90 has less affinity concerning the organs of the reticulo-endothelial system and no influence can be observed on the number of lymphocytes in the peripheral blood, whereas the gold-198 colloid lowers the number of lymphocytes to one third of the initial value (Scheer, 1956). Attention has been focused in the past fifteen years on the radiation hazard of the lanthanide rare earths and of yttrium because of the high yields with which they are produced in the fission of uranium or plutonium. At least ten radiolanthanons and six radioisotopes of yttrium (Kyker, 1962) are formed. Since they are generally beta-emitters with associated gamma-radiation, and since their half-lives ordinarily do not exceed a few hundred days, they have not been classed with the most hazardous isotopes in the production of chronic poisoning. However,

for a relatively short time after fission their contribution to the total radiation level is highly significant (Norris et al., 1956).

Toxicologic evaluation of the lanthanons in mammals had little systematic attention prior to the studies with lanthanum and yttrium in rats done by Cochran et al. (1950). These workers compared the oxides with certain soluble salts of these elements by oral and intraperitoneal routes. The oxides were much less toxic, 10-20 times more oxide being tolerated orally than was true for ordinary salts. The intraperitoneal LD_{50} of lanthanum salts ranged from 134 to 209 mg. of the element per kilogram of body weight as follows (in order of descending toxicity): sulfate, nitrate, ammonium nitrate, chloride, and acetate. By weight, the toxicity of yttrium was higher, but on a molar basis it was slightly lower. In rats, Kyker and Cress (1957) found a somewhat higher toxicity for yttrium and lanthanum chlorides when administered intraperitoneally. Mice, on the other hand, tolerated almost twice as much yttrium as rats.

Magnusson (1963) determined the acute toxic effect of injected yttrium, cerium, terbium, holmium, and ytterbium on the rat liver by the following means: 1) studying their influence on the blood glucose level, 2) investigating the extent of acute liver damage by determination of ornithine carbamyl transferase (OCT) in the blood serum, and 3) confirming the acute liver damage morphologically by macroscopic, light

microscopic, and electron microscopic methods. According to
the results, the amount of glucose decreased soon after ad-
ministration for all elements and as a rule the glucose levels
were within the normal limits of variation four days after ad-
ministration. The OCT concentration in the blood serum in-
creased during the first days after administration with all
of the above metals. These levels were also within the normal
limits four days after injection. In regard to macroscopic
and microscopic lesions, the light lanthanons produced a dif-
fuse fatty degeneration in the liver which, with identical
dosing, is demonstrable only in female rats. The heavy lan-
thanons and yttrium produced focal necrosis in the liver which
can be seen in female as well as male rats. Toxic lesions
were usually distributed uniformly throughout the liver which
supported the opinion that the lesions could be ascribed to a
direct toxic effect of the metal on the liver cells.

In the electron photomicrographs of livers from the
yttrium, terbium, holmium, and ytterbium injected rats, the
cells showed a variable picture. Cells which seemed to be
normal and cells which showed various grades of injury were
seen. The mitochondria showed a slight degree of enlargement.
Within limited areas the endoplasmic reticulum was lacking
occasionally in ribosomes. A dilatation of the cisternae of
the endoplasmic reticulum was seen frequently. The cerium
injected females showed severe changes in all organelles.
The above observations suggested that the toxicity of the

lanthanons is decreased with a rise in atomic number. This finding had also been observed by Kyker and Cress (1957). The toxicity of the lanthanons was also shown to decrease with rising atomic number when given by intraperitoneal injection (Graca et al., 1962). Lanthanons are usually divided into light and heavy groups. To the former belong lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, and gadolinium; and to the latter terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutecium. Magnusson (1963) further observed that an appreciable difference prevails between the toxicities of the light and heavy lanthanons. He states that as far as the question of toxicity is concerned, yttrium is not like the heavy lanthanons with which it is similar in many respects because his work indicates that yttrium is more toxic than the heavy lanthanons. It should be pointed out that the dosage used for yttrium was approximately one fourth to one sixth of that for the heavy lanthanons. Also the dose for cerium was approximately one twelfth to one twentieth of that for the heavy lanthanons.

An insight into the behavior of yttrium when once within the animal organism is provided by Ramsden (1961). She did an extensive literature review on the tissue distribution, mechanism of deposition in bone, and the state of the injected radioyttrium in the blood. This review concluded that with regard to tissue distribution yttrium is a

bone-seeking isotope when injected in a carrier-free form. In
the presence of the carrier, aggregates of larger particle size
are formed, and these show greater affinity for the soft tis-
sues which have the highest proportion of reticulo-endothelial
cells. Chelating agents render the yttrium more soluble, thus
increasing excretion. They reduce the extent of colloid for-
mation and reduce the size of the colloidal particles formed.
The site of deposition depends on the particular chelate and
its concentration, but it can be predicted that the concentra-
tion of yttrium in soft tissues will be higher than in the
case of carrier-free radioyttrium. As regards the mechanism
of deposition in bone, yttrium, unlike the alkaline earths,
appears to be taken up on resorbing or quiescent bone sur-
faces where calcification is not taking place. It is also
present diffusely throughout mineralized bone. The mechanism
of deposition is possibly one of surface adsorption. The
state of yttrium in the blood was concluded by stating that
electrophoretic studies of yttrium-91 in plasma, both in vitro
and in vivo, show that it remains at the point of application
indicating the absence of an ionized fraction. On the other
hand, electrophoretic studies of serum have shown a migrating
yttrium-91 band between the alpha- and beta-globulins, a non-
migrating fraction which is of colloidal appearance in auto-
radiographs, and advanced evidence for phospholipid binding
of yttrium in the migrating fraction. Yttrium protein com-
pounds may be complex salts in which the metal is only

partially in a readily ionizable form or chelates of high stability which do not readily dissociate free yttrium ions. As mentioned previously, these conclusions were based upon injected radioyttrium.

The elimination of radioisotopes from the lung, including radioyttrium, was briefly reviewed by Schubert (1951). He stated that following the inhalation of smokes, aerosols, or dusts containing radioisotopes, the material appears in the lungs and the upper respiratory tract. The cilia of the nasopharynx, the lower part of the vestibule of the larynx, the trachea, and especially the large bronchioles propel material toward the mouth where it is swallowed, ultimately appearing in the feces. In addition to ciliary action, the peristaltic motion in the bronchioles, the cough reflex, and the nasal hair remove significant amounts of material. In addition to these, phagocytic cells transport particles to the ciliated surfaces of the respiratory bronchioles where they are subsequently removed by ciliary action. Material not removed mechanically can leave the lung in two ways. The foreign particles may be engulfed by phagocytes and carried into the lymphatic system of the lung by migration through the alveolar lining, or they may be dissolved and absorbed into the blood stream. Movement and distribution within the lung are largely functions of particle size. The chemical properties of yttrium indicate that within the respiratory passages it would react as colloidal or particulate material.

Vorwald and Willard (1963) exposed sixty-six rats to an aerosol of yttrium-91 oxide for one exposure of 30 minutes. Exposure in this experiment was "nose-only" with anesthetized rats. Four rats were euthanized and necropsied at 82 days and other rats at intervals throughout life. Following the necropsy of 27 rats they observed that, calculated as μc/gm of tissue, the lung retained a relatively high concentration reaching a plateau at eight weeks. The next highest concentrations observed in descending order were for skin, bone, and kidney. No information was given for other organs.

Davison and Talbot (1965) injected stable yttrium oxide in a carboxymethylcellulose carrier via trachea into the lungs of rats and guinea pigs. At 60, 90, and 120 days following the injection, animals were euthanized, various organ wet weights obtained, various organs ashed, and the yttrium content determined by mass emission spectrographic methods. When related to the per cent of total dose injected, the lung contained the greatest amount followed by femur, spleen, and liver in that order. In this respect, it would appear that translocation to other organs following lung exposure behaves similarly to injected carrier-free radioyttrium.

Present knowledge of the internal behavior of rare earths and yttrium in living systems has come almost exclusively from the use of the radioisotopes of these elements (Kyker, 1962).

The excretion of intravenously administered yttrium has been studied utilizing radioyttrium. In tracer-solutions,

radioactive isotopes occur in a low chemical concentration. Under certain conditions these have a strong tendency to form radiocolloids (Schweitzer, 1956). Magnusson (1963) injected various radiolanthanons and yttrium-91 at pH 3.0 to 3.5. At this pH he assumed that he had a highly ionized solution. The blood concentrations dropped much more rapidly when in colloidal form than in ionized form. He noticed that the serum concentration one hour after administration of yttrium-91 was only 0.30 per cent of the given dose per ml. The serum concentration after two and four days was almost the same for yttrium-91, cerium-144, promethium-147, and ytterbium-169. Schubert et al. (1950) report about the same blood concentration for yttrium-91. The rapid fall of the amount of yttrium in the blood has been emphasized by Kyker et al. (1954). Magnusson (1963) further observed that yttrium-91 and terbium-160 appeared in higher amounts in the kidney than the other lanthanons investigated. Yttrium-91 showed the largest total excretion level with about five per cent of the given dose appearing in the urine during the first two to three hours after injection. Of the materials investigated (yttrium-91, cerium-144, promethium-147, terbium-160, holmium-166, and ytterbium-169) yttrium-91 occupied a unique position in that its uptake by the liver was lower than that of the other elements. The maximum content of yttrium-91 in the liver was 25-30 per cent of the given dose. This value was obtained after both two and three hours. Some days after the intravenous injection, the

liver contained about ten per cent of the given dose of yttrium-91. Durbin et al. (1956) had demonstrated that the rare earth metals are excreted, among other routes by way of the gastrointestinal tract. Magnusson (1963) further demonstrated that the presence in the gastrointestinal tract was due to excretion by way of bile. As per the total dose injected, the amount excreted by bile was least for yttrium-91 and the most for holmium-166. When a maximum excretion was reached, a rapid drop in further excretion was observed with yttrium-91 followed by a slow rise. This suggested a stronger binding to the liver cells as was also true of the light lanthanons investigated. It was also postulated that excretion occurred directly through the gastrointestinal tract. It is known that this occurs with serum albumin and since the rare earth metals and yttrium are transported in the blood while bound to albumins (Ekman et al., 1961), a gastrointestinal excretion appears reasonable.

The subcellular distribution of injected yttrium-91, cerium-144, promethium-147, terbium-160, holmium-166, and ytterbium-169 in rat liver cells was also investigated by Magnusson (1963). All of the above could be demonstrated in the various cell fractions each time a sample was taken. The nuclear fractions had the lowest amount of the investigated nuclides. The mitochondrial fraction showed an uptake of all nuclides, and it occurred in both sexes. The microsome

fraction had a constant uptake. The initial high amounts of all the lanthanons and yttrium in the supernatant following administration decreased with time. This suggests that the lanthanons and yttrium are probably first deposited in the cytoplasmic matrix of the liver cell followed by transportation to the various cellular organelles.

Inhalation of Yttrium and Certain Rare Earths

Little information has been reported on the effects of inhalation of the stable rare earths and yttrium. The first account appears to be that of Schepers et al. (1955). Intra-tracheal injections of two blends were administered to two separate groups of nine guinea pigs. Equal amounts of each blend were given to the respective groups at seven-day inter-vals. One blend was composed of 31% carbon, 39.6% rare earth fluorides, 26.4% rare earth oxides and 3% potassium sulfate. The second blend consisted of 17% carbon, 3% graphite, 65% rare earth fluorides, 10% rare earth oxides and 5% potassium sulfate. For the intratracheal injections a 10% suspension in isotonic saline was made in which particles were 3μ and under in diameter. The dose administered was 100 mg. The guinea pigs to which the blends were administered either died or were sacrificed at varying time intervals up to 540 days after the first injection. An inhalation study utilized 75 guinea pigs with the latter blend. For this study the exposure period was for eight hours daily, five and one-half days per week,

and for three years. The calculated particle size was stated to be one to two microns in diameter and concentration was expressed as 2.0 to 3.0 x 10^8 particles per cubic foot. During the three year exposure period, 25 guinea pigs died of various causes. The remaining 50 were sacrificed at various intervals throughout the three year period. For both groups, inhalation and intratracheal, the gross observations indicated that these two blends acted as relatively inert substances. No gross pathological changes could be observed in the experimental animals indicating any form of pneumoconiotic fibrosis. For the inhalation group, progressive accumulation of the rare earths in the lungs was confirmed by chemical analysis. At the end of three years the rare earths constituted approximately 50% of the lung ash wherein the ash comprised approximately 7% of the dry tissue.

Schepers (1955a) studied the histopathology produced by the intratracheal administration of the blend containing a relatively high oxide content. He noted that a fatal, delayed, chemical hyperemia was produced in one third of the animals injected. In those that survived the introduction of the dust, cellular eosinophilia was a prominent feature. Most of the dust was trapped within focal atelectatic areas, and no appreciable chronic cellular reaction or fibrosis occurred in relation to these deposits. Isolated cellular vascular granulomas were found after one year. Most of the pigment seemed to be transported gradually to the hilar lymph nodes, but it

caused no significant reaction.

Schepers (1955b) further studied the histopathology pro-
duced by a blend having a relatively high fluoride content
administered both intratracheally and by inhalation. He con-
cluded that when introduced by intratracheal injection, a
blend of rare earth compounds dominantly composed of fluo-
rides, provokes an acute transient chemical pneumonitis, sub-
acute bronchitis, and bronchiolitis. Residual deposits were
present to which no reaction occurred. The dominant histo-
pathological changes, which result progressively from the pro-
longed inhalation of the rare earth high-fluoride dust, com-
prise focal hypertrophic emphysema, regional bronchiolar
stricturing, and subacute bronchitis. Pigment is focally re-
tained, but it provokes no fibrosis or granulomatosis. It
was pointed out for blend no. 1 that there was too little po-
tassium sulfate in this blend (3%) to have played a significant
role as an inhibitor of fibrosis, but the carbon (31%) may
have reduced any intrinsic pathogenicity which the rare earths
may have possessed. Also, the ratio of rare earth fluorides
to oxides would nullify any sclerosing influence of either
component and that such leads need to be investigated.

Davison (1963) reported on a critical controlled inhala-
tion study of the pathogenesis of neodymium oxide in mice and
guinea pigs. In this experiment approximately 60 per cent of
the mass of the dust generated was contained in particles less

than 1.0 μ in diameter. A chamber concentration of 30 mg. neodymium oxide/meter^3 of air was maintained for periods of six hours per day for five days per week. Animals were taken off test after every ten exposure days. The longest cumulative 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.

Davison (1963) found that gross and histological changes revealed a progressive impaction of neodymium in the lungs. It was contained primarily in macrophages and some neodymium was transported to the tracheobronchial lymph nodes where it could be seen after 100 days in the mice and 50 days in the guinea pigs. Lymphocytic hyperplasia was common, but it was not considered to be a specific response to neodymium. Mechanical disruption of the normal architecture of the

pulmonary parenchyma was usual after 60 days of exposure. This resulted from the heavy deposition of the foreign material. There was 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.

The exposed male guinea pigs did not gain as rapidly as the male controls. It was supposed that this may have resulted from the fact that exposed males were more restless and fought more than the control males. The other organs, glands, and hematologic parameters remained normal. 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 (Davison, 1963).

Vorwald and Forest (1963) exposed albino rats and guinea pigs to stable yttrium oxide at concentrations of 72 mg./ft^3. Groups of rats were given single exposures for 5, 15, and 30 minutes; multiple exposures (6, 12) for five minute periods and 18 exposures of five and 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 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 ex-
periment have shown an increasing amount of particulate matter
with persistence of a nodular reticulo-endothelial hyperpla-
sia in the tracheobronchial lymph nodes. Lung changes were
mild and consisted of some early septal fibrosis and occa-
sional epithelial hyperplasia in guinea pigs. Exposure in
this experiment was a "nose-only" exposure with anesthetized
rats. There was no information supplied concerning the par-
ticle size of the yttrium used.

Talbot et al. (1964) reported on a study wherein 120 CFW
white mice, equally divided between sexes, were exposed to
gadolinium oxide at a concentration of 30 mg./meter^3 of air
for periods of six hours per day and five days a week. The
longest duration of exposure was 120 days. At the end of
each 20-day exposure period, ten males and ten females were
taken off test. At the same time, an equal number of control
mice which had been under the same atmospheric and stress con-
ditions, with the exception that no gadolinium oxide had been
introduced into their test chamber, were taken off test. A
progressive impaction of gadolinium in the lungs was observed
as time of exposure increased. The material could not be seen
in the tracheobronchial lymph nodes until after 100 days of
exposure. There were no acute inflammatory reactions and no

fibrosis, but simply a thickening of the alveolar wall and an accumulation of dust-laden macrophages. Other organs were normal, and hematologic parameters were not significantly different than the control mice values.

Lung Clearance

Hatch and Gross (1964) state that alveolar clearance is that process by which the alveolar surface is kept free of exogenous and endogenous insoluble matter which could constitute a barrier to free gaseous interchange. They also state that there are two pathways by which lung tissue may be cleansed of entrapped matter: one leading to the pharynx and the other to lymph nodes. The first is physiological and by far the most effective in terms of the amount of matter removed. The second has pathological implications and generally involves a minor fraction of the total material that comes to rest upon the alveolar surface. There are two main components to the first process of alveolar clearance: 1) phagocytosis of the particulates and 2) transport of the cells and material to the moving mucous blanket. It is also recognized (Hatch and Gross, 1964) that a dust particle settling upon the alveolar surface may be sequestrated within the lung by a tissue reaction (pneumoconiosis) or be dissolved and transferred in solution to the blood stream. The former is dependent upon an inadequacy of the clearance mechanism in association with other factors. The latter is limited by the solubility and

solution rate of the particles.

The role which phagocytosis plays in the hygiene of the alveoli is as follows:

1) To render dust particles incapable of irritating or otherwise injuring the alveolar surface epithelium.

2) To prevent the penetration of the dust particles into the lung interstitium.

3) To facilitate the removal of the dust by the alveolar clearance mechanism.

The first report on electron microscopy of the lung was published by Low (1952). His conclusions pointed to the existence of a continuous alveolar epithelium, "with cell bodies located chiefly on the thicker portions of alveolar walls and attenuated to form a complete lining." A year later Low (1953) described uninterrupted basement membranes localized along the basal surfaces of both alveolar epithelium and capillary endothelium. The pulmonary alveolar cells which form the covering of the alveolar surface and which Macklin (1954) called "pneumonocytes" are of two varieties distinguishable on the basis of structural and functional differences. Bertalanffy et al. (1953) distinguished a larger granular pneumonocyte and a smaller endothelium-like cell. Hatch and Gross (1964) state that the phagocytic cells are the large alveolar cells derived from the alveolar surface epithelium. They further state that for many years a number of investigators maintained that alveolar cells were not phagocytic while still attached

to the alveolar wall, but this opinion is no longer appropriate. These cells generally desquamate, however, soon after they have ingested dust particles, but the mechanism which brings this about is not known. The desquamated cells, usually termed "alveolar macrophages" or "dust cells", may continue their phagocytic activity for sometime within the alveolus (Hatch and Gross, 1964).

Casarett (1960) states that many investigations attempting to demonstrate the origin of macrophages in interstitial regions utilized intratracheal injection or the use of materials which were irritative, inflammatory, or infectious. Under such circumstances, it is now recognized that migration from interstitial regions of phagocytic cells might occur to a degree which could easily mask any contribution of the alveolar epithelium to the pool of phagocytic cells in the exudate.

With the electron microscope, Karrer (1958) observed phagocytosis in the lung as a process associated with plasma membrane infolding and the formation of vesicles. Later (Karrer, 1960), a variant of such a simple invagination process was suggested in which large "flaps" or "ruffles" might trap "large" volumes of extracellular material as the "ruffles" backfold upon the cell. The resulting inpocketings are subsequently subdivided into smaller vacuoles and vesicles as the backfolded "ruffle" fuses at different points with the cell surface. Karrer (1960) states that phagocytosis and

pinocytosis obviously are closely comparable processes, distinguished only on the basis of the ingested material. It is solid in the case of phagocytosis and fluid or semifluid in the case of pinocytosis.

Lymphatic transport of dust is properly considered a component of alveolar clearance because the removal of dust from points of initial deposition, upon or within the alveolar wall, is involved (Hatch and Gross, 1964). But in order to be transported by way of lymphatic vessels, the dust particles must first penetrate the alveolar membrane or otherwise gain access to the interstitium. Gross and Westrick (1954) state that there are two concepts in regard to the mechanism by which the dust particles may penetrate the alveolar membrane. One assumes that phagocytes transport the dust particles across the membrane and then into the lymphatics, and the other theorizes that the dust penetrates the alveolar membrane as naked particles which are carried as such by tissue fluid to the lymphatics. The tenet that alveolar dust particles are carried through the intact alveolar wall by phagocytic cells assumes the existence of an unknown and undescribed tropism. Further, this phenomenon of desquamated alveolar cells re-entering tissues represents a unique performance on the part of cells which have been largely deprived of their nutrition and damaged by ingested noxious particles. Therefore, the concept of direct penetration of dust particles was favored

over phagocytic transport by Hatch and Gross (1964). Karrer
(1960) demonstrated an alveolar macrophage with ingested India
ink particles within the septum between two alveoli. He pos-
tulated that ultimately the free phagocytes seem to enter al-
veolar septa and that probably they will eventually reach the
lymph channels, an assumption based on numerous histopatholog-
ic observations. Casarett and Milley (1964) suggested that
alveolar epithelial cells are phagocytic and serve as at least
one source of alveolar macrophages contributing to clearance
of inhaled material. They also suggest that alveolar epithe-
lial cells, having phagocytized material, move either into
alveolar spaces or into interstitial tissue depending, in
part, on the relation of the cell to adjacent cellular
components.

Response of the Body to Exercise

Guyton (1964) states that while the body is at rest, the
skeletal muscles, which comprise over one half of the body,
receive only 15% of the total blood flow. However, it is
pointed out that during exercise quite a different situation
is presented for essentially all of the increase in blood flow
occurs in the muscles. This increases as much as 15-fold
during very intense exercise representing as much as 75% of
the total blood flow. Guyton (1961) emphasizes that during
strenuous exercise the diffusing capacity for oxygen in the
lungs increases about three times the diffusing capacity

under resting conditions. It is stated that this increase is caused by three different factors:

1) Opening up of a number of previously dormant pulmonary capillaries, thereby increasing the surface area of the diffusing membrane.

2) Dilation of all the pulmonary capillaries that were already open, thereby increasing the total surface area.

3) Possibly also opening up some previously atelectatic alveoli.

Therefore, during exercise, the oxygenation of the blood is increased not only by increased alveolar ventilation but also by a greater capacity of the respiratory membrane for transmitting oxygen into the blood. It is apparent that the body has a tremendous compensatory mechanism for supplying oxygen to the muscles during periods of strenuous exercise.

In general, carbohydrates are broken down aerobically, i.e., with a supply of oxygen. Anaerobic glycolysis seems to be more of a bypass for cells with a poor oxygen supply (Karlson, 1963). Baldwin (1963) states that it is characteristic of the anaerobic metabolism of muscle that glycogen is broken down and lactate formed. Lactate arises by the production of pyruvate, and its formation provides a mechanism for the reoxidation of reduced nicotinamide adenine dinucleotide (NAD). Unless the reduced coenzyme was in some way reoxidized, no further production of glyceric acid phosphate could take place and the generation of new high energy phosphate for the resynthesis of adenosine triphosphate would come to an end.

Baldwin (1963) further states that under aerobic conditions, the reduced coenzyme is rapidly reoxidized through the flavoprotein/cytochrome/cytochrome oxidase reaction chain and no lactate need be produced at all.

Liljestrand and Wilson (1925) cite the work of Fletcher and Hopkins (1907) which demonstrates conclusively that lactic acid is formed in muscle during contraction. Lactic acid is rapidly diffusible and uniformly distributed through the body. The concentration of lactic acid in the blood is proportional to the amount of lactic acid in the body at that time (Margaria et al., 1933). Karpovich (1959) reports that the accumulation of lactic acid depends on the relative intensity of the exercise, and that the normal content of lactic acid in the blood of man is about ten mg. per 100 cc. Taylor (1944) reports readings as high as 300 mg. per 100 cc. for man. After strenuous exercise is discontinued, lactic acid continues to escape from the muscles into the blood. The period of two to eight minutes immediately after strenuous exercise marks a high unchanging level of lactic acid in the blood after which it begins to decline and reaches pre-exercise levels in 30 to 90 minutes. Yoder et al. (1964) used blood lactate determinations as a parameter in the evaluation of physical fitness in dogs. Blood for lactate analysis was obtained two minutes after a predetermined exercise period on the treadmill once weekly for six weeks. Nine dogs were subjected to the test

exercise. Average weekly results of all blood lactate tests demonstrate a rapid lowering of the lactate level beginning with the first week and extending to the second week with subsequent minor fluctuations during the remainder of the conditioning period. The analysis of variance showed that lactate levels after exercise decrease during conditioning with a significant difference among the first three weeks in a downward direction. Baldwin (1963) points out that in mild or moderate exercise oxygen is brought into the cells fast enough to re-oxidize reduced NAD as rapidly as it is formed. Thus, little or no lactate is produced, and pyruvate, instead of being reduced, is completely oxidized. Further, if the degree of exertion is increased, glycogen will be more rapidly broken down and NAD proportionately more rapidly reduced. Eventually, with increasing severity of exercise, a point will be reached at which the oxygen supplied by the circulatory apparatus can just keep pace with the reduction of the coenzyme. But the muscle can work still harder by utilizing oxygen as fast as it is made available by the circulatory system and by reoxidizing any coenzyme that still remains in the reduced condition by using the anaerobic device of lactate formation. It can thus be seen that alterations in the lung can impair the diffusibility of oxygen, and alterations in the blood can reduce the oxygen carrying capacity which would tend to invoke the anaerobic mechanism for reoxidation of NAD. Injury to

various organelles within the cells conceivably could result in altered enzyme activity of the tricarboxylic acid cycle and electron transport chain thus further invoking the anaerobic route for reoxidation of NAD. Hettinger et al. (1961) state that during prolonged, heavy, physical work the individual's capacity for performance depends largely on his ability to take, transport, and deliver oxygen to the working muscle. Therefore, one might expect that during a period of daily treadmill exercise an increase in total hemoglobin might be evident.

Knehr et al. (1942) studied men in training over a period of six months and found no significant differences in hemoglobin concentration. Robinson et al. (1937) checked various athletes and found the hemoglobin concentration like that of untrained men.

Davis and Brewer (1935) exercised four dogs by swimming (two hours daily) or by treadmill exercise (25% grade, six miles daily, speed not given) and found that blood volume was markedly decreased in the first week of exercise in three of the four dogs. All dogs after six weeks of exercise showed a blood volume higher than normal. The hemoglobin per unit volume of blood was lowered significantly in all four dogs during the first week, but it recovered during the exercise period and in no case exceeded the normal level. However, the total circulating hemoglobin was increased significantly

by five to seven weeks of exercise.

Yoder et al. (1964) pointed out that there appeared to be gross changes in hemoglobin (hemoglobin/kg. body weight) values as a result of variation in body weight, hemoglobin concentration, and blood volume occurring during a training period. These changes might have been attributed to the effects of chronic exercise had it not been for a parallel reaction in the control animals.

Steinhaus (1933) stated the belief that training brings about an increase in the per cent of hemoglobin, the total mass of corpuscles, and the total volume of blood, rests on meager experimental data. The work of Thörner (1932) was cited showing that trained dogs had a reduction in hemoglobin as compared with untrained littermates.

Tsuchiya (1936) exercised four groups of dogs as follows:

Group A. Exercised for two hours daily for 14 days continually on the treadmill inclined at 11 degrees.

Group B. Exercised for three hours daily for 14 days continually on the treadmill inclined at 11 degrees.

Group C. Exercised for 4 hours daily for 14 days continually on the treadmill inclined at 11 degrees.

Group D. Daily rapid running for 12 days was instituted on the horizontal treadmill until fatigued.

Three dogs were in each group. The velocity of running was not given inasmuch as it was so regulated that speed was adjusted to each individual. Dogs unable to run the fixed

distance and the required number of days were discarded. A greater reduction in hemoglobin occurred with more rapid running (Group D) while all groups showed a decrease during the relatively short exercise period. In general, the hemoglobin concentration paralleled the changes in erythrocyte numbers.

Results obtained by the various experiments appear to vary with the stress imposed by the experiment, length of experiment, type of experiment, and condition of experimental subjects. The early red cell destruction is probably attributed to, as suggested by Steinhaus (1933), a weeding out of older, less resistant corpuscles by the fragmentation process which is augmented by the higher circulatory rates from exercise. A return to normal or higher values would then be evident because, as suggested by Broun (1923), the hematopoietic tissues have adapted themselves to the increased demands made upon them by the hastened rate of destruction.

MATERIALS AND METHODS

Experimental Animals

All dogs used were beagles of approximately the same age within each experiment and of approximately the same weight within each sex. They were of good conformation. All dogs were purchased from Morris Research Laboratories, 4000 Meriden Road, Topeka, Kansas. The dogs for each experiment were ordered approximately six months in advance of their anticipated need so that uniformity could be attempted. In many cases littermates were obtained.

Prior to their shipment, all dogs were vaccinated for canine distemper, infectious canine hepatitis and leptospirosis with Virogen DHL.[1] This was done far enough in advance so that immunization was assumed to be complete prior to shipment.

Upon arrival, all dogs were assigned to cages and later tattooed for permanent identification. In addition to feeding, watering, cleaning, and obtaining the rectal temperature, the dogs were observed closely during the first 7-10 days. The 24 dogs remained well throughout the experiments. A complete physical examination was given to all dogs. They were checked for intestinal parasitism and wormed with Vermiplex[1] and Whipcide,[1] if indicated. All dogs were examined for Dirofilaria immitis according to the method described by Newton

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[1] Allied Laboratories, Indianapolis, Indiana.

and Wright (1957). All dogs were negative for this parasite.

When the dogs were not being used on experiment, they were confined to galvanized metal cages,[1] measuring 28" x 36" x 30", in an air-conditioned room.

The dogs were fed Purina Dog Chow[2] once daily following their removal from the chambers in the evening. They were fed according to the directions of the manufacturer and more particularly to the needs of the dog so that obesity did not develop. Water was supplied ad lib.

Body weights were obtained and recorded weekly. Appetite and behavior were observed daily and recorded if abnormal.

The specifications for each dog and whether or not it was exposed or served as a histologic control during the treatment period are listed in Table 1.

Experimental Design

Each of three separate experiments (8A, 8B, 8C), utilized eight beagle dogs, four males and four females.

A gradual period of training for the dogs to run on the treadmill then commenced. Following the training period, a 10-week experiment was started. Treadmill exercise was given between 10:00-12:00 a.m. for 10 minutes daily per dog for five days per week throughout the experiment. The dogs were placed in individual cages and the cages placed in a chamber

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[1]Harlan Industries, Cumberland, Indiana.

[2]Ralston Purina Company, St. Louis, Missouri.

Table 1. Specifications of dogs for experiments 8A, 8B, and 8C

Experiment	Dog no.	Sex	Age on arrival (months)	Body weight prior to treatment period (lbs.)	Body weight after treatment period (lbs.)	Designation
8A	31	M	6	27.5	27.3	Exposed
	32	M	6	21	22.5	Exposed
	33	M	6	26.8	26.3	Exposed
	34	F	6	20.8	21.3	Exposed
	35	F	6	16	17.8	Exposed
	36	F	6	18	19.3	Exposed
	37	M	6	32	30.5	Control
	38	F	5	22.3	23	Control
8B	39	M	11	24	24.5	Control
	40	M	6	20.8	21	Exposed
	41	M	6	21.5	21.3	Exposed
	42	F	9	21.5	21.5	Exposed
	43	F	11	21	21.3	Control
	44	F	9	22.3	22.3	Exposed
	45	M	9	25	25.3	Exposed
	46	F	11	21.5	21.8	Exposed
8C	47	M	7	35.3	37	Exposed
	48	M	8	25.8	26	Exposed
	49	M	8	23.5	23.3	Exposed
	50	M	9	26.5	27	Control
	51	F	8	19.8	20.5	Exposed
	52	F	8	17	16	Control
	53	F	9	20.3	19.5	Exposed
	54	F	9	24.3	24.5	Exposed

environment each afternoon for six hours daily, five days per week for the 10 week experiment.

The animals were in a paired experiment in which the first four weeks served as the control period and the next six weeks as the treatment period. During the first four weeks each dog experienced all of the routines and the procedures with the exception of the addition of yttrium oxide to the chamber atmosphere. The last six weeks served as the treatment period when yttrium oxide was added to the chamber atmosphere.

With this design it was possible to establish a "normal" prior to the introduction of yttrium oxide and to obtain a series of observations during the inhalation or treatment period. The various responses could then be expressed as "change" over the treatment period. To provide for histologic comparisons, two dogs, one male and one female, from each group of eight were placed in chambers in which yttrium oxide was not introduced during the six-week treatment period. Blood lactate was determined twice weekly throughout the experiment following treadmill exercise. Red cell volume, plasma volume, hemoglobin concentration, and white blood cell enumeration were determined prior to yttrium oxide exposure and at the end of the six-week treatment period. A radiographic examination was made prior to exposure and again at the end of the treatment period.

During the treatment period a daily sample of dust was

obtained from the chamber atmosphere for the determination of yttrium oxide concentration. Twice weekly, samples were obtained from the chamber atmosphere with a cascade impactor to determine the mass distribution of yttrium oxide. The stages of the cascade impactor were then characterized as to particle size distribution so that some quantitation of dosage could be obtained.

Prior to necropsy of the dogs, each dog was anesthetized and the oxygen consumption was determined so that the volume of air inhaled for any period of time could be calculated for dosage considerations. The dogs were euthanized and a necropsy of each dog was performed. Tissues harvested for histologic examination were lung, bronchial lymph nodes, liver, kidney, spleen, testis or ovary, adrenal gland, heart muscle, mesenteric lymph node, and sternum.

Treadmill Training

All treadmill exercising was done in an air-conditioned room wherein the temperature was maintained at 72-74 F. The treadmill[1] (Figures 1 and 2) had a variable-speed electric motor so that any speed between 0 and 10.75 miles per hour could be attained. The running plane could be adjusted from 0-20 degrees at five degree intervals. An iron frame was affixed towards the front end of the treadmill so that the

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[1]Designed and constructed by Iowa State University Instrument Shop, Ames, Iowa.

Figure 1. Treadmill with speed control unit.

Figure 2. Treadmill with dog in running position.
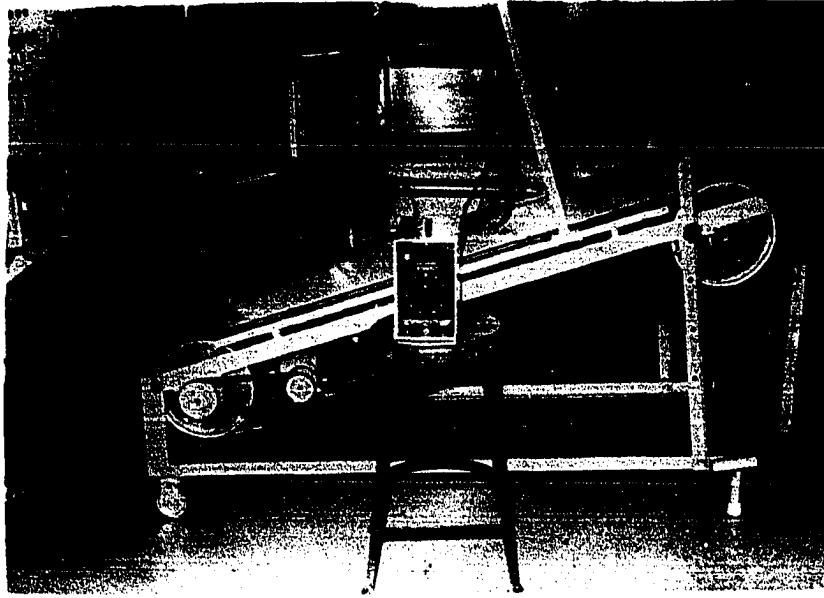
harness chain could be attached and thereby offer some guidance for the dog. The dog was placed on the treadmill and a regular dog harness was put on. The harness was preferred over a collar so that obstruction to air flow would not occur when the dog was unable to keep up with the treadmill.

Initial training consisted of allowing the dog to stand on the treadmill with the harness on for 10-15 minutes per day for three to four days. Following this the treadmill belt was put in very slow motion so that the dog could become accustomed to walking on the treadmill. At this point the dog would often hang back in the harness. By coaching and sometimes pulling forward on the harness the dog would begin to walk. The speed and incline of the treadmill were gradually increased so that by the end of four to five weeks, training was considered accomplished. At this point the experiment would be started and further increases in receptivity and running ability would be noticed. In no case would the treatment phase of the experiment begin without some assurance that training ability had reached a plateau.

It was observed that there is a tremendous difference in dogs as to their willingness to exercise on the treadmill. The dogs which appeared receptive and unafraid of the trainer would in most instances be good runners from the start. Others through continued encouragement and training eventually became good runners. However, in each group of 8 dogs, there would be one or two which never became good runners

and would tend to hang back in the harness. The latter dogs were accordingly assigned to be histologic controls inasmuch as their blood lactate levels were not meaningful.

Establishment of Test Exercise

It was important to establish a fixed speed for each dog so that any changes noted in blood lactate concentration could be attributed to reasons other than the amount of work output. The speed, incline, and duration also had to be such that it represented a good work output for that dog. Yoder et al. (1964) in working with dogs of mixed breeding and variable size established a maximal speed for a fixed subject. They determined the stride frequency (number of times right fore-foot is brought forward/minute) for said fixed subject and proceeded to calculate a speed for the remaining dogs by determining their stride frequency and substituting this value in the following equation:

$$\frac{\text{stride frequency of fixed subject}}{\text{stride frequency of subject } y} \quad x \quad \text{fixed subject's speed}$$

$$= \text{rate in m.p.h. of subject } y$$
$$\text{(to nearest tenth mile)}$$

The duration of their test was arbitrarily fixed at 10 minutes.

In experiment 8A an attempt was made to simulate this method. Inasmuch as our dogs were of relatively uniform size, very little difference could be detected in stride frequency

and it was decided to run all subjects at the same speed, incline, and duration. The speed control unit was set so that the belt speed was 4.27 m.p.h., the incline was set at 20 degrees, and the length of the test was set at 10 minutes.

It became apparent that the running abilities of the individuals were not closely paralleled, but since all subjects appeared to have a fairly good workout, no change was made because the treatment phase had started. While this experiment was still in progress, an attempt was made to determine more accurately the stride frequency by use of slow motion photography. The results of this experiment indicated that the minor adjustments which would accordingly be made in the speed would not adequately adjust the differences observed in running ability.

It was decided that individual adjustments in speed, depending upon the dog's ability to run, would be attempted in the two subsequent experiments (8B and 8C). The incline and duration would remain at 20 degrees and 10 minutes, respectively, for all dogs and the speed would be set at a point where the blood lactate reached a plateau preferably in the range of 15 to 30 mg. %. This seemed logical in that any blood lactate increase which might be observed later would be relative to the pre-existing level. A speed was then reached which appeared to represent a good work output for each individual and which correlated with a plateau of blood lactate

concentration.

In experiment 8C the dogs appeared less willing to run at an incline of 20 degrees so the angle was decreased to 15 degrees and the test speed obtained.

The various speeds of the dogs in the three experiments are shown in Table 2.

Table 2. Speed and inclination of treadmill and duration of daily run for all dogs in experiments 8A, 8B, and 8C

Experiment number	Inclination (degrees)	Duration of daily run (minutes)	Dog number	Speed (m.p.h.)
8A	20	10	31	4.27
			32	4.27
			33	4.27
			34	4.27
			35	4.27
			36	4.27
			37	4.27
			38	4.27
8B	20	10	39	5.79
			40	4.41
			41	4.00
			42	5.79
			43	5.79
			44	5.50
			45	5.79
			46	5.79
8C	15	10	47	5.79
			48	6.61
			49	5.79
			50	5.24
			51	5.51
			52	4.96
			53	4.96
			54	5.24

Method of Exposure

The exposure and control chambers were those for which design requirements had been set by Davison (1963) and for which the design was accomplished and described by Cook (1961). The large chamber (Figure 3) was used for the six dogs where exposure to yttrium oxide occurred in the last six weeks of each experiment. The small chamber (Figure 4) was used for the two dogs which served as histologic controls in each experiment where exposure to yttrium oxide was not wanted. While in the chambers, the dogs were confined to individual stainless steel cages[1] measuring 9" wide x 15-1/2" high and 23" long. The top, bottom, sides, and ends were constructed of 3/8 inch expanded stainless steel fabric so that free passage of air could take place.

Aerosol Preparation and Generation

The yttrium oxide[2] used for these experiments was guaranteed 99.9% pure. It was received as a finely pulverized powder of unknown particle size. This product was further reduced to a moderately uniform and satisfactory mean particle diameter by two stages. In the first stage the oxide was

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[1]Constructed by Iowa State University Instrument Shop, Ames, Iowa.

[2]Purchased from Rare Earths and Thorium Division, Michigan Chemical Corporation, St. Louis, Michigan.

Figure 3. Inhalation chamber used for dogs where exposure
.to yttrium oxide was accomplished.
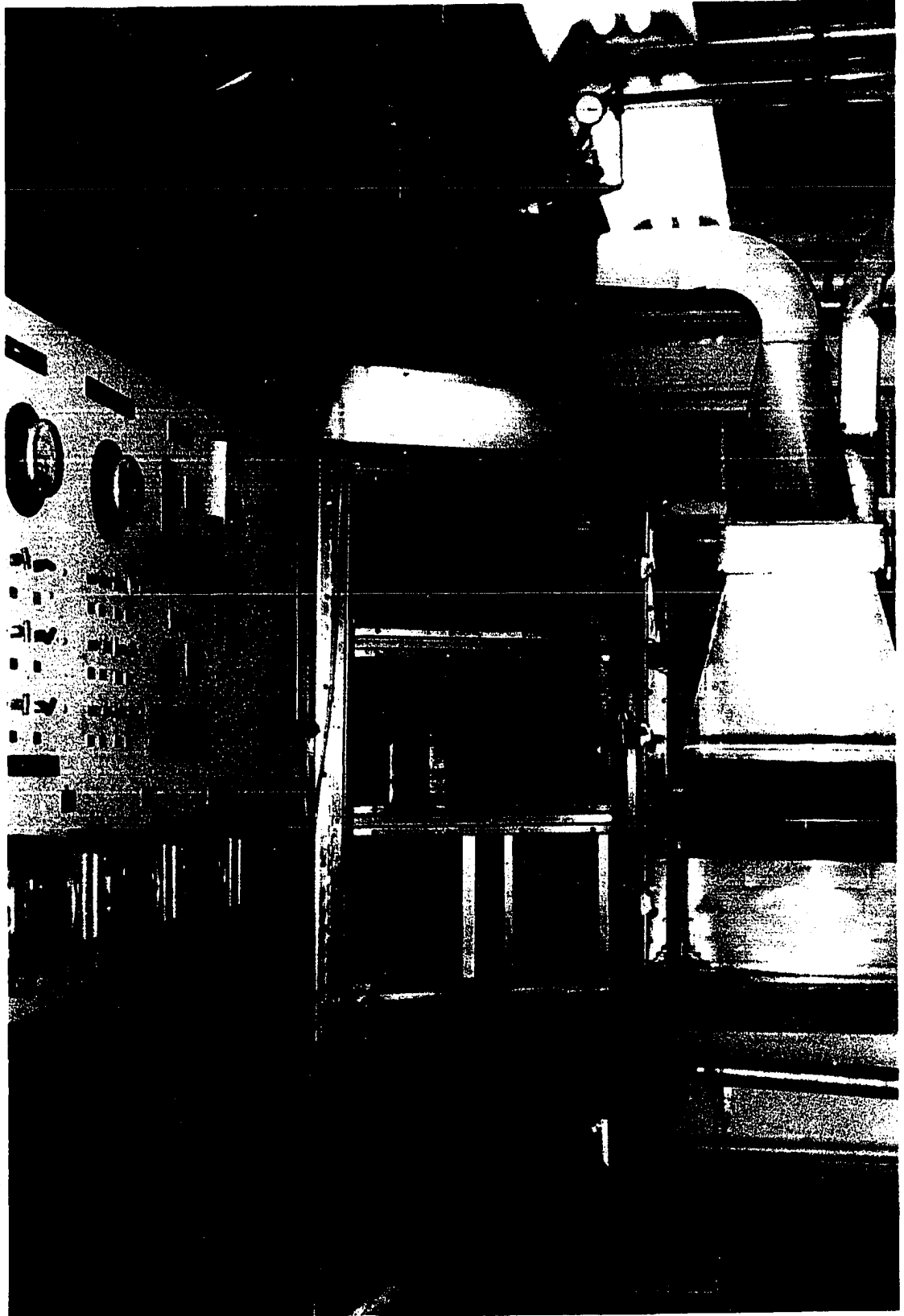
further pulverized in a Pica Blender Mill[1] using stainless
steel vials and balls for eight minutes. The second stage
consisted of manually regrinding the powder, suspended in
absolute alcohol, in a mortar with a ceramic pestle. This
second stage further reduced the particle size. The alcohol
was removed from the mixture by burning, and a satisfactory
dry dust remained. This yttrium oxide dust was then stored
in a desiccator until it was used.

Generation of the aerosol was accomplished with a Wright
dust feed mechanism[2] (Figure 5). Its operation was dependent
upon a stream of air removing collected dust as it was scraped
from a revolving cylinder tightly packed with the yttrium
oxide. The amount of dust generated was dependent upon the
gear ratio of the mechanism rotating the dust packed cylinder.
The proper gear ratio was obtained by trial and error. The
dust was introduced at the top of the chamber in such a manner
that incoming air gave an even distribution in all parts of
the chamber as demonstrated by Cook (1961).

Concentration of aerosol in the chamber was dependent
upon the gear ratio of the Wright mechanism, the air pressure
used to remove the collected dust from the scraper, and the
air-replacement rate in the chamber. Variation in tightness

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[1]Pica Blender Mill, Model 3800, Pitchford Scientific
Instruments Corporation, Pittsburgh, Pennsylvania.

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

Figure 4.   Inhalation chamber used for dogs where no yttrium
            oxide was introduced into the chamber atmosphere.

Figure 5.   Wright dust-feed mechanism used for generation of
            the aerosol.
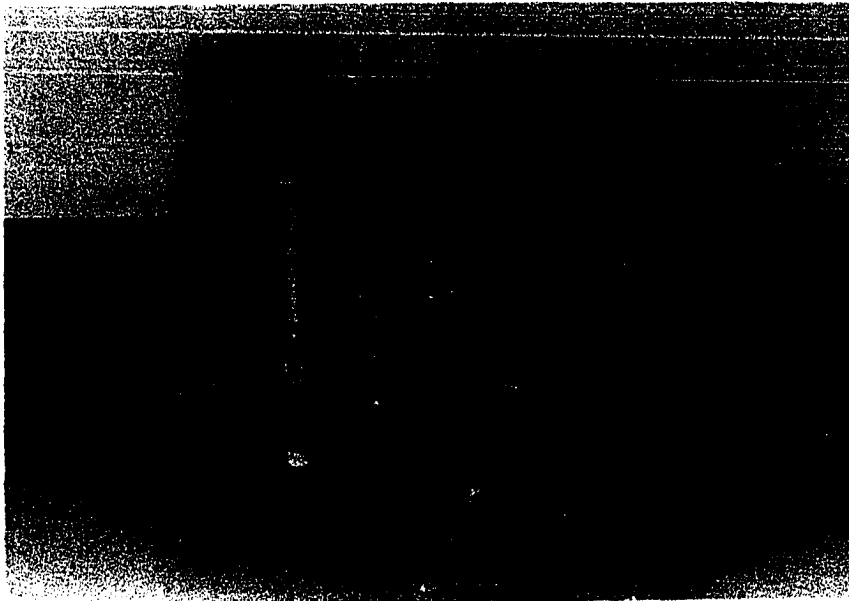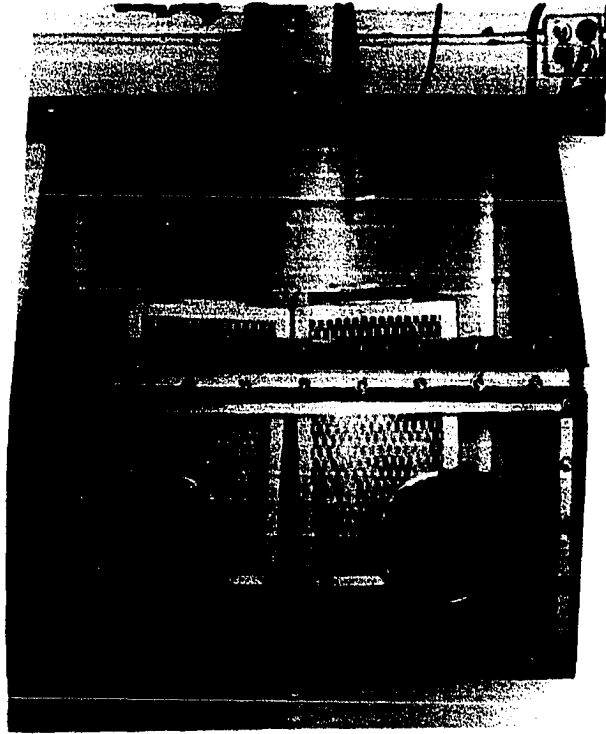
of cylinder pack necessitated occasional changes of the gear ratios. An air-replacement rate of 8 ft.^{3}/min. and an air pressure at the scraper blade of 5 pounds/inch^2 provided the proper dispersal of yttrium oxide.

Dehumidification of the air to the Wright dust feed mechanism was necessary to prevent clogging of the scraper plate. This was accomplished by a water trap at the air compressor unit and by an air filter and dehumidifying unit in the line before the air passed the scraper blade.

Aerosol Concentration

The desired chamber concentration of yttrium oxide during the exposure period was arbitrarily set at 15 mg./meter^3. In order to maintain a closeness to this desired concentration, daily sampling was required and adjustments made accordingly.

Daily sampling was accomplished by drawing 16.8 ft.^{3}/hr. of chamber air across a cellulose ester filter[1] for 15 minutes. The filter was held in a stainless steel open end filter holder which was affixed to the end of a stainless steel tube. This allowed for sampling from about the middle of the chamber. Manipulations of the filter holder and tube were accomplished through a capped hole in the plexiglass side of the chamber.

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[1]MF type DAWP25, Millipore Filter Corporation, Bedford, Massachusetts.

The method described by Fritz et al. (1958) was used to determine the amount of yttrium oxide on each filter. This method uses arsenazo [3-(2-arsonophenylazo)-4,5-dihydroxy-2,7-napthalene-disulfonic acid] as the color-forming reagent for the quantitative determination of yttrium and the rare earths. The method is applicable to the determination of very low concentrations of the above and is not highly pH dependent.

In this experiment the yttrium oxide was eluted from the filter with two separate washings of 10 ml. of hot, dilute nitric acid (1:1). The solution from each washing was transferred to a 50 ml. volumetric flask, the beaker quantitatively rinsed with ion free water into the volumetric, and the flask taken to volume with ion free water. A 1 ml. aliquot of this dilution was used for the determination. The sample was buffered with a triethanolamine buffer, purified arsenazo solution added, and the pH adjusted to 8.2 with ammonium hydroxide. The resultant color was measured against a reagent blank, prepared with an unexposed filter, at 570 mμ in a spectrophotometer[1]. Results were reported as mg/meter^3 of air.

Mean concentrations, standard deviations, and 99% confidence limits for the mean on 30 samples from each experiment are listed in Table 3.

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[1] Coleman Jr. spectrophotometer, Coleman Instruments, Inc., Maywood, Illinois.

Table 3. Chamber atmosphere concentration means, standard deviations, and 99% confidence intervals for means

Experiment number	Mean mg.Y_2O_3/meter^3	s^a	Confidence intervals[b]
8A	20.63	3.03	19.11 - 22.15
8B	12.67	5.04	9.45 - 15.89
8C	16.88	3.88	15.18 - 18.58

[a]Standard deviation.

[b]Ninety-nine per cent confidence intervals for means.

Particle Size Determination

The fraction of an inhaled dust which is retained in the respiratory system and the depth to which the dust penetrates before deposition are closely related to its particle size. Thus, the size factor is a primary one in determining the degree of hazard associated with the inhalation of particulate matter. Specifically, in the case of silicosis and other pulmonary dust diseases, interest is limited to that portion of the total inhaled material which is deposited in the alveoli. Dautrebande et al. (1957) state that there is common agreement that large dust particles settle onto the mucosa of the upper respiratory tract and as the particles become smaller, they penetrate more deeply into the lungs. They further emphasized that retention of particles below 1.2 μ in the upper regions of the respiratory tract is

practically zero.

Morrow (1960) reviewed several physical factors controlling the fate of inhaled substances. For particles larger than 0.5μ, the dominant force for deposition is gravitational settling. Particles less than 0.1μ diameter are dependent upon Brownian motion for impaction. There is a misconception that the range between 0.1 and 0.5μ represents a rather stable aerosol and is not subject to deposition. Morrow (1960) pointed out, however, that the above data were derived from unit density particles and that when working with oxides of heavy metals the density must be considered and that deposition of particles in the range of $0.1 - 0.5\mu$ is in fact a reality. Density is not involved in deposition caused by Brownian movement. Morrow (1960) 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 fate of the large particles (greater than 5μ) is probably of little consequence. Moreover, the relatively high deposition and retention of small particles (less than 1μ) in the lung parenchyma have been repeatedly demonstrated.

Dygert et al. (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. They reported that

particles larger than 1.0 μ were less toxic than those smaller than 1.0 μ.

Davison (1963) estimated that 60% of the generated neodymium oxide dust was contained in particles less than 1.0 μ in diameter. This was the quantity that was considered to permit deposition in the terminal bronchioles and alveoli. From these figures, dosages were estimated which gave an index of the amount of material to which each animal was exposed.

It is apparent that dosage of inhaled materials is difficult to determine and can only be approximated. In this experiment, the per cent of the total mass of yttrium oxide less than 1.0 μ in diameter was determined. This mass was then considered to be of the proper characteristics to permit deposition in the terminal bronchioles and alveoli. It was realized that a small percentage of particles greater than 1 μ might also be deposited but their quantitation was not attempted.

Chamber atmosphere was sampled with a point-to-plane electrostatic precipitator[1]. Samples were precipitated directly on a parlodion coated, 200 mesh, copper, electron microscope grid which had been overlaid with a carbon film

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[1]Precipitator constructed by Iowa State University Instrument Shop, Ames, Iowa. Power source for precipitator designed and constructed by Mr. A. O. Stattelman formerly of Iowa State University.

Precipitation was accomplished by drawing chamber air through the instrument at a flow rate of 2.5 ft.^{3}/hr. A voltage of 7 KV producing 4-5 microamperes of current across the air gap for 7 seconds 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. The maximum diameter horizontal with the top or bottom edge of the electron photomicrograph was determined with a particle size analyzer[1].

Further analysis of data obtained from the analyzer gave a mean particle diameter and standard deviation of 0.393 $\pm$ 0.345 μ. Ninety-nine per cent confidence limits for the mean were $\pm$ 0.024 μ. Examination of data showed that the particle diameter distribution was logarithmically normal, and all calculations were based on this type of distribution. These figures were based upon 627 particles photographed at random from the grid and represented that portion of the population that could be seen and measured at a magnification of 10,000 x.

Mass Distribution of Aerosol

In order to determine what per cent of the total mass of aerosol generated consisted of particles less than 1 μ, the mass distribution of aerosol was determined. A Casella

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[1]Particle Size Analyzer, Model TGZ-3, Carl Zeiss, Oberkochen/Wuertt., West Germany.

cascade impactor[1] was utilized for this purpose. 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 increase from jet to jet when air is drawn through at a steady rate. Between the fourth stage and the line to the vacuum pump is a filter unit. The largest particles are removed at the first stage, the next largest at the second stage and so on until those particles too small for impaction at the fourth stage are trapped on the filter. The jets were designed so that line deposits are obtained. The flow rate of collection was adjusted to 17.5 liters/minute which gave velocities through the four jets of 2.2, 10.2, 27.5, and 77 meters per second, respectively. When distribution samples were collected, glass coverslips coated with stop-cock grease were affixed to the stages. The same type of filter as was used for sampling the chamber concentration was placed in the filter unit. The stop-cock grease allowed for retention of the impacted particles. Samples were collected for 30 seconds. This minimum time was desired so that impacted particles would not tend to pile up and cause deflection of subsequent particles. The method as described for determining chamber aerosol concentration was

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

used for the quantitative determination of yttrium oxide impacted at each stage and the filter. Hot, dilute nitric acid (1:1) melted the stop-cock grease and thus released the particles so that they would go into solution. Upon cooling, the stop-cock grease solidified and was retained on the sides of the beaker used for putting the yttrium oxide in solution. Accordingly, it did not interfere with the remainder of the test. A total of 22 samples were collected in this manner and it was determined that 73.86 per cent of the mass was collected at stage 3, stage 4, and the filter.

It was also necessary to know the size of the particles which were collected on the various stages. For this determination, electron microscope grids were mounted in the line of deposition at each stage and particles were impinged directly on these grids for counting. Air flow through the cascade impactor was started prior to placing it in the chamber atmosphere. Sampling for three seconds from the chamber atmosphere was found to be optimal for impacting large numbers of particles with a minimum of piling. It was found that the mass of the particles collected at stages 1 and 2 was sufficient to break the parlodion film and carbon overlay which had been applied to the grids. This method was adequate for stages 3 and 4. The method of particle size analysis was the same as that used for samples collected on the electro-static precipitator except that approximately

3000 particles per stage were analyzed. Mean diameters and standard deviations for stages 3 and 4 were found to be 0.5796 ± 0.5088 μ and 0.3721 ± 0.2903 μ, respectively. Samples from stage 2 were collected on a vaseline coated coverslip and examined under the light microscope. The use of a calibrated eyepiece indicated that these particles were 5-6 μ in diameter. Because of their relatively large size (greater than 1 μ) this portion of the mass distribution (approximately 26%) was not included for dosage considerations. A summary of mass distribution and particle size representation for each stage of the Casella cascade impactor appears in Table 4.

Dosage Calculation

In order to arrive at an estimate of dosage, the air intake of the dogs exposed to yttrium oxide had to be determined. This was obtained by determining the oxygen consumption rate of each dog and converting this value to volume of air breathed. The oxygen contents of inspired air and expired air were assumed to be 20.93 and 16.29%, respectively (Dukes, 1955). The difference of 4.64% was used in the conversion of oxygen consumption rate to volume of air breathed.

The oxygen consumption rate for each dog was determined with the aid of a spirometer[1]. This apparatus has a closed

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[1]Recording Metabolor, Model 175, McKesson Appliance Co., Toledo, Ohio.

Table 4. Yttrium oxide mass distribution and particle size representation for each stage and filter of Casella cascade impactor

Stage number	Mass distribution (per cent)			Particle size (microns)		
	mean	s[a]	confidence interval[b]	mean	s[a]	confidence interval[b]
1	2.17	2.03	0.93-3.39	not determined		
2	23.97	7.89	19.21-28.73	>1		
3	48.81	7.05	44.55-53.07	0.5796	0.5088	0.5616-0.5976
4	20.41	7.67	15.78-25.04	0.3721	0.2903	0.3624-0.3818
Filter	4.64	3.30	2.65-6.63	not determined		

[a]Standard deviation.

[b]Ninety-nine per cent confidence interval for the means.

system which was flushed and filled with oxygen. It contained soda lime for absorption of carbon dioxide in the exhaled air. Each dog was anesthetized[1], an endotracheal tube with cuff was inserted, cuff inflated, and the endotracheal tube attached to the metabalor. The oxygen consumption was determined for a 30 minute period. The oxygen consumed (ml.) and volume of air (ml.) respired per kilogram of body weight per hour were calculated for each dog and are presented in Table 5.

Morrow (1960) states that it has been necessary for certain "Committees" to create formulations upon which they hope conservative estimations of inhalation hazard can be based. As an outstanding example of this he cited the so-called Harriman Conference model which was applicable to man. A model similar to this was followed to estimate the dosage for each of the dogs exposed to yttrium oxide on this experiment.

From the oxygen consumption studies it was determined that the average dog breathed 7.45 liters of air per kilogram of body weight per hour. For experiments 8A, 8B, and 8C there was an average of 20.63, 12.67, and 16.88 mg; respectively, of Y_2O_3/meter^3 of air available. During the 30-day exposure period (180 exposure hours) each dog would have breathed 1.34 meter^3 of air per kilogram of body weight. The particle size-mass relationship estimated that 74% of this dust was of a

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[1]Nembutal Sodium (Pentobarbital Sodium, Abbott), Abbott Laboratories, North Chicago, Illinois.

Table 5. Oxygen consumption converted to volume of air respired for beagle dogs on experiments 8A, 8B, and 8C

Exp. no.	Dog number	Body weight (kg.)	Oxygen consumption (ml./kg./hour)	Air respired (liters/kg./hour)
8A	31	12.4	299	6.444
	32	10.2	303	6.530
	33	11.9	293	6.315
	34	9.7	352	7.586
	35	8.1	335	7.220
	36	8.8	411	8.858
	37	13.9	354	7.629
	38	10.5	354	7.629
8B	39	11.2	464	10.000
	40	9.5	337	7.263
	41	9.7	495	10.668
	42	9.8	306	6.595
	43	9.7	557	12.004
	44	10.2	402	8.664
	45	11.5	400	8.621
	46	9.9	485	10.453
8C	47	15.9	252	5.431
	48	11.7	308	6.638
	49	10.8	259	5.582
	50	11.8	356	7.672
	51	9.2	348	7.500
	52	7.5	134	2.888
	53	9.2	130	2.802
	54	10.9	312	6.724

Mean of air respired = 7.45 liters/kg./hour.
Standard deviation = 2.09 liters/kg./hour.

character that would permit deposition in the terminal bron-
chioles and alveoli. From these figures, dosages could be
estimated which gave an index of the amount of material to
which each dog was exposed. The values derived are presented
in Table 6.

Postexercise Blood Lactate

Blood samples for lactate determination were obtained
twice weekly during each experiment. To allow for equal
spacing between samples and also to overcome any influence of
weekend inactivity on the dogs, it was decided to obtain sam-
ples on Tuesday and Friday of each week.

Each sample consisted of 5 ml. of blood withdrawn directly
into a syringe containing 10 to 15 mg. crystalline ammonium
fluoride. The ammonium fluoride prevents coagulation and also
stops the conversion of blood glucose to lactate on standing.
The blood was withdrawn from the right or left jugular vein
approximately 30 seconds after the established treadmill exer-
cise had been completed. Analysis for lactate was done the
same day that the sample was obtained.

The method of Barker and Summerson (1941) was used for all
lactate determinations. The step-wise procedure for this
method was prepared by Fister (1950). In this method 1 ml. of
blood was deproteinized and the glucose and other interfering
material of the protein-free blood filtrate were removed by
treating with copper sulfate and calcium hydroxide. An aliquot

Table 6. Estimated dosage of inhaled yttrium oxide (based on an air consumption of 7.45 liters/kg./hr. or 1.34 meters^3/kg./180 exposure hours)

Exp. no.	Mean chamber concentration (mg.Y_2O_3/meter^3)	Dog no.	meters^3 air breathed	mg. Y_2O_3 available in air breathed	mg. Y_2O_3 available to lung[a]	dose[b] (mg./kg.body wt.)
8A	20.13	31	16.62	343	253	20.4
		32	13.67	282	209	
		33	15.95	327	242	
		34	13.00	267	198	
		35	10.85	223	165	
		36	11.79	241	178	
8B	12.67	40	12.73	160	118	12.4
		41	13.00	164	121	
		42	13.13	166	123	
		44	13.67	173	128	
		45	15.41	194	144	
		46	13.27	166	123	
8C	16.88	47	21.31	359	266	16.6
		48	15.69	264	195	
		49	14.47	243	180	
		51	12.33	207	153	
		53	12.33	207	153	
		54	14.61	246	182	

[a]Based on 74% availability of total mass due to particle size distribution.

[b]Based on assumption of 100% retention.

of the resulting solution was heated with concentrated sulfuric acid to convert lactic acid to acetaldehyde. This was then treated with p-hydroxydiphenyl[1] in the presence of copper ions to produce a condensation product with a purple color. The transmittancy of the samples was measured against a reagent blank set at 100% transmission at a wave length of 565 mμ. The concentrations were then obtained from a previously prepared lactic acid standard[2] curve.

Erythrocyte Volume

A modification of the method of Sterling and Gray (1950) as described by Talbot (1963) was used for measuring the circulating erythrocyte volume. For each determination on eight dogs, 45 ml. of blood were withdrawn aseptically from a donor dog into a syringe containing 10 ml. of a sterile anticoagulant solution[3]. Approximately 175 micro-curies of sterile $Na_2Cr^{51}O_4$[4] were added to the blood and the mixture incubated for 30 minutes at 37°C. The unbound chromium was then reduced

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[1]p-phenylphenol, Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, N. Y.

[2]Lactic acid standard obtained from Hartman-Leddon Company, Inc., 60th and Woodland Avenue, Philadelphia 43, Pa.

[3]Special Formula ACD solution obtained from Abbott Laboratories, Oak Ridge, Tennessee.

[4]Rachromate obtained from Abbott Laboratories, Oak Ridge, Tennessee.

from the anionic hexavalent form to the cationic trivalent form with 2 mg. ascorbic acid per 1 ml. blood. The latter form does not penetrate the erythrocytes and eliminates the possibility of labeling erythrocytes *in vivo*. The red cells were washed two times in sterile 0.9 per cent NaCl solution and resuspended in 0.9 per cent NaCl solution. A counting standard was prepared by diluting 1 ml. of the labeled blood to 250 ml. with distilled water. One ml. of this dilution was pipetted into a counting tube for determination of radioactivity.

The labeled blood was administered to each dog by injection into the cephalic vein. Five ml. (approximately 20 microcuries) were given to each dog. When the erythrocyte volume was determined at the end of each experiment, a blood sample was obtained prior to injection of each dog. This was used to determine if there was any count remaining from the previous injection. When present, it was subtracted from the count obtained postinjection.

After allowing 20 minutes for mixture of the labeled cells throughout the circulatory system, a 10 ml. blood sample was obtained from the jugular vein. Seven ml. were allowed to clot for T-1824 dye analysis and the remaining three were transferred to a tube containing edetate (ethylenediamine-tetraacetic acid) as the anticoagulant. One ml. from the latter was transferred to a counting tube containing 1 ml. of distilled water to accomplish hemolysis. The remaining 2 ml. were used for other hematological determinations, which

included PCV (packed cell volume).

Determination of the radioactivity present was made by counting the samples in a well-type, thallium activated sodium iodide crystal scintillation counter[1]. Natural radiation present was determined from an average of three separate determinations obtained on the day of counting. This was then subtracted from the count obtained from the samples. The activity present in each sample was counted to a preset count of 10,000 in order to reduce radiation statistical probability error and the activity then recorded as counts per minute per ml. of cells by the appropriate calculations from the PCV. The circulating erythrocyte volume was calculated as follows:

$$\frac{(\text{counts/min./ml. standard}) \ (250) \ (\text{ml. injected})}{\text{counts/min./ml. cells}}$$

= Circulating erythrocyte volume (ml.)

No correction of the PCV for the plasma trapping factor was made because the microhematocrit method of determination was used and a greater packing of cells is obtained. Further, since the difference in erythrocyte volume was sought, between the start of the exposure and the termination, any correction factor would be relative.

In Experiment 8C, the terminal erythrocyte volume was determined initially using blood which had been obtained from a donor dog and subsequently mixed with $Na_2Cr^{51}O_4$. It was

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[1]Nuclear-Chicago Corporation, 333 E. Howard Avenue, Des Plaines, Illinois.

observed in four of the eight dogs injected with the above that a very low postinjection count was obtained. It was assumed that agglutination of the donor cells had occurred. Four days later the erythrocyte volume was again determined using autologous blood. The postinjection counts and calculated erythrocyte volumes appeared normal on this latter determination. The agglutinating factor was not determined.

Plasma Volume

The plasma volume for each dog was determined by modifications of the T-1824 dye dilution method of Gregerson (1944) and described by Allen and Semple (1951) and Talbot (1963).

The T-1824 dye[1] was prepared in a one per cent solution from the same lot for the entire study. A tuberculin syringe with needle attached was weighed before and after the injection of an estimated amount of T-1824 solution into the cephalic vein. In this manner, the exact amount injected could be determined. Twenty minutes were allowed for complete mixing in the circulatory system. The 7 ml. venous blood sample which was allowed to clot was then centrifuged for 15 minutes at 2800 R.P.M. and the serum harvested for T-1824 dye extraction. The dye was extracted from the serum by the method of Campbell et al. (1958).

After extraction of the dye, the concentration in mg. per

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[1]Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, N. Y.

ml. of serum was determined spectrophotometrically. A Coleman Jr. spectrophotometer[1] with matched cuvettes was used for all spectrophotometric determinations of dye concentration. It was assumed that the excretory rate of the dye was 0.1% per minute (Gregerson and Rawson, 1959) and accordingly the quantity which was excreted prior to obtaining the blood sample was calculated. The formula used to calculate the plasma volume was as follows:

Plasma Volume (ml.) =

$$\frac{\text{Amount of dye injected (mg.) - amount of dye excreted (mg.)}}{\text{Concentration of dye in serum (mg./ml.)}}$$

Other Hematologic Parameters

Leukocyte enumerations were determined utilizing National Bureau of Standards certified pipettes, counting chambers and cover glasses. The diluting fluid used was 0.1 N HCl.

The packed cell volume was determined by the microhematocrit capillary tube method. The capillary tubes were filled with fresh blood from blood samples where coagulation was prevented with edetate and centrifuged[2] for five minutes. Readings were made in a microhematocrit reader[2].

The total hemoglobin concentration was determined using the cyanmethemoglobin method as described by Leavell and Thorup (1960). Conversion of the hemoglobin in blood to

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[1]Coleman Jr. spectrophotometer, Coleman Instruments, Inc., Maywood, Illinois.

[2]Clay Adams, Inc., New York, N. Y.

cyanmethemoglobin was accomplished with Drabkin's Solution[1]. The transmittancy of the resulting solution was determined with a spectrophotometer[2] set at 540 mμ and compared with the transmittancy of the standard[1].

Macroscopic Studies

When each experiment was terminated the dogs were anesthetized[3] and then euthanized by severing the axillary artery and vein. The latter procedure allowed for exsanguination. Necropsy examinations were then performed and all organs were examined for gross changes.

Microscopic Studies

Portions of sternum, lung, heart, liver, spleen, adrenals, kidneys, bronchial lymph nodes, gonad and mesenteric lymph node were obtained at the time of necropsy for histologic examination. These tissues were fixed in 10% formalin. Paraffin sections were cut at a thickness of 6μ and stained with hematoxylin-eosin.

A slight modification of the usual tissue harvest and fixation technique for the lungs was performed in Experiment

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[1]Cyanmethemoglobin Reagent and Cyanmethemoglobin Certified Standard purchased from Hycel, Inc., 230 Medical Arts Building, Houston, Texas.

[2]Coleman Jr. spectrophotometer, Coleman Instruments, Inc., Maywood, Illinois.

[3]Nembutal Sodium (Pentobarbital Sodium, Abbott), Abbott Laboratories, North Chicago, Illinois.

8C. Following their removal at necropsy with the trachea intact, 10% formalin was infused by gravity flow via the trachea. Expansion of the lung did not exceed what was considered to be a normal inspiration. The trachea was ligated and the tissue samples were removed and transferred to 10% formalin about 30 to 40 minutes after infusion.

Radiographic Examination

An X-ray examination of the thoracic region was performed on each dog before exposure to yttrium oxide and at the termination of the exposure period. The examination prior to exposure rendered valuable assistance in determining the freedom from lung pathology and subsequent fitness for treadmill running. It was also of interest to note if any increase in radiographic density might occur as a result of 30 days (180 exposure hours) exposure to yttrium oxide. Ventro-dorsal and left lateral exposures were obtained on 10" x 12" film[1]. A Patterson Par Speed screen[2] was used for each exposure. The X-ray machine[3] was set at 10 Ma. The film-target distance was 32.5 inches and exposure was for 0.2 second. Voltage was selected according to the following formula (Hutchinson and

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[1] Kodak Medical X-ray Film (Blue Brand), Eastman Kodak Company, Rochester, New York.

[2] E. I. Du Pont de Nemours and Company, Bloomington 98, Delaware.

[3] Mobile X-ray unit, Model D-3, General Electric Company, Schenectady, N. Y.

Clark, 1964):

$$KvP = 2 \times thickness \ (cm) + factor$$

factor = 35, 40, or 45 for low, medium or high
density tissues, respectively.

For the thoracic region, the factor of 35 was used.

RESULTS AND DISCUSSION

Blood Lactate

The blood lactate determinations were made throughout the experiment. The values are presented graphically in Figures 6 through 23 for each dog. The difference in blood lactate for each dog was obtained by subtracting a value representing the "normal" for that dog at the beginning of exposure from a value representing the dog at the termination of 30 days exposure (180 exposure hours) to yttrium oxide. The normal values were obtained by averaging four determinations (two from the week prior to exposure and two from the week after exposure began). These four values in most cases represented the most consistent plateau. The values representing the dogs at the termination of exposure were obtained from an average of the last four determinations (last two weeks of exposure). The differences obtained are presented in Table 7. The mean of the difference was +5.00 mg./100 ml. of blood. An analysis of variance was performed and is presented in Table 8. Application of the F-test (Snedecor, 1956) indicates that there is no significant difference due to replications and sex. The difference in blood lactate for the overall experiment was tested with Student's t-test (Snedecor, 1956) according to the following equations:

$$t = \frac{\bar{d} - o}{\sqrt{\frac{MS_E}{18}}}$$

$$d.f. = 14$$

$$t_{.05} = 1.761$$

Figure 6. Postexercise blood lactate levels for dog 31. Cal-
            culated difference between beginning and termina-
            tion of exposure to yttrium oxide is -6.0 mg./100
            ml. (The values representing the dog at the be-
            ginning were obtained by averaging four determina-
            tions, two from the week prior to exposure and two
            from the week after exposure began. The values
            representing the dogs at the termination of ex-
            posure were obtained from an average of the last
            four determinations.)

Figure 7. Postexercise blood lactate levels for dog 32. Cal-
            culated difference between beginning and termina-
            tion of exposure to yttrium oxide is +4.0 mg./
            100 ml.

EXPERIMENT 8A
DOG 31

EXPERIMENT 8A
DOG 32

Figure 8. Postexercise blood lactate levels for dog 33. Calculated difference between beginning and termination of exposure to yttrium oxide is +1.0 mg./ 100 ml.

Figure 9. Postexercise blood lactate levels for dog 34. Calculated difference between beginning and termination of exposure to yttrium oxide is -7.0 mg./ 100 ml.

EXPERIMENT 8A
DOG 33

EXPERIMENT 8A
DOG 34

Figure 10. Postexercise blood lactate levels for dog 35. Calculated difference between beginning and termination of exposure to yttrium oxide is +2.0 mg./100 ml.

Figure 11. Postexercise blood lactate levels for dog 36. Calculated difference between beginning and termination of exposure to yttrium oxide is +26.0 mg./100 ml.

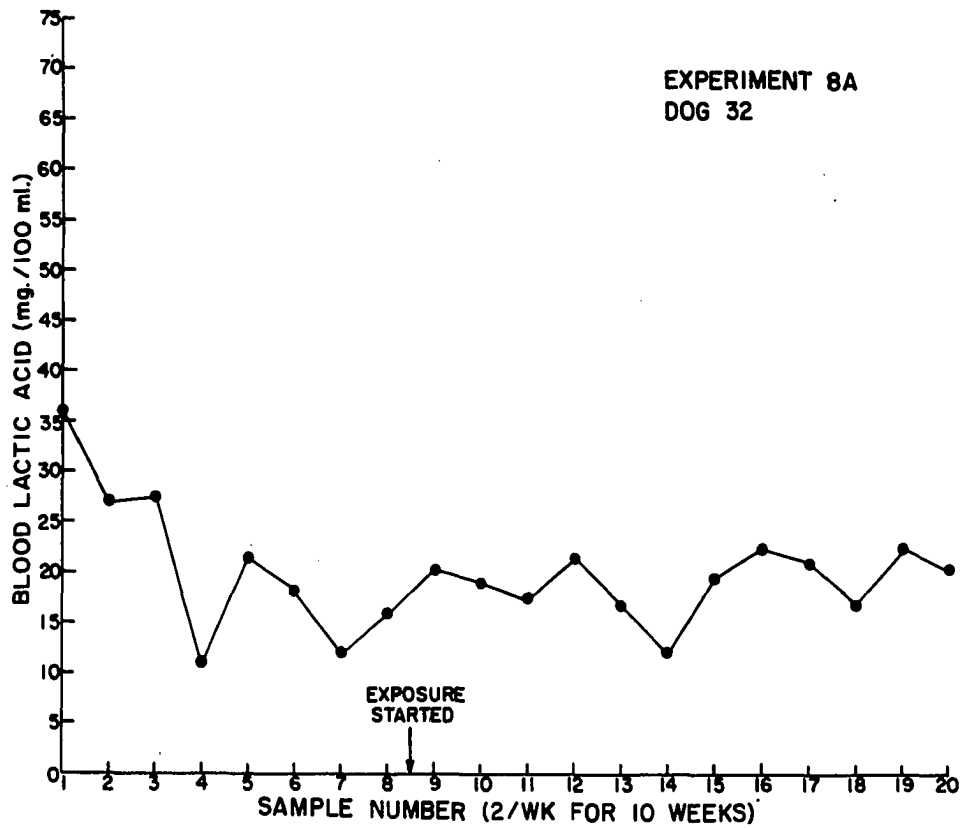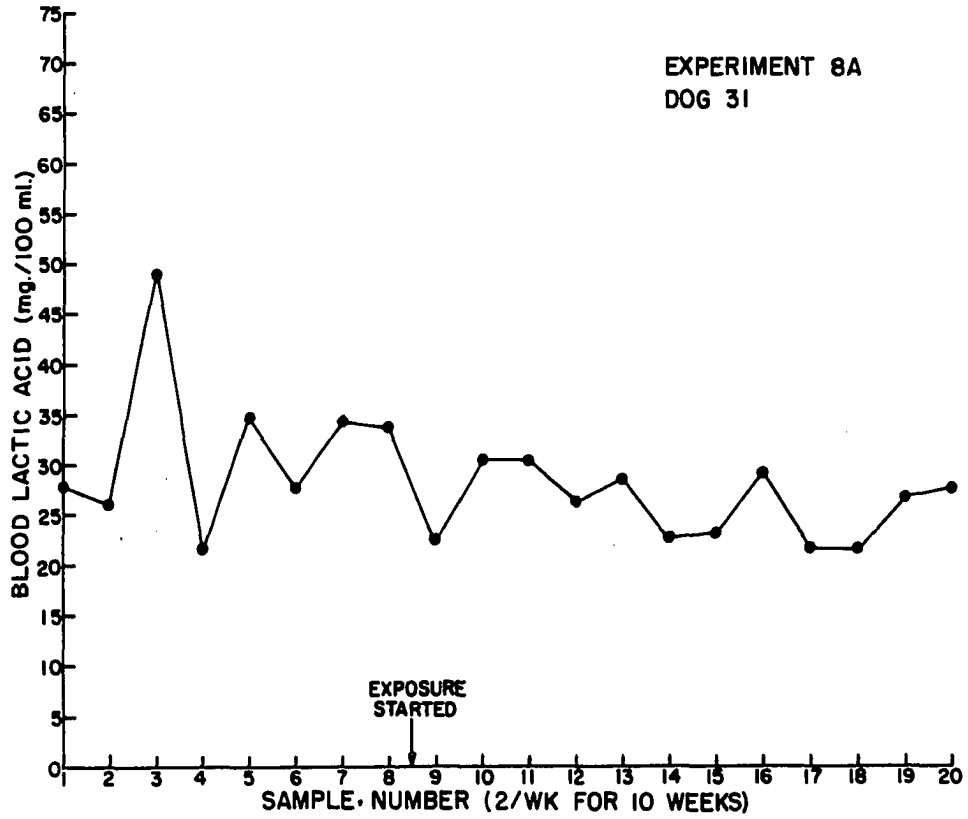EXPERIMENT 8A
DOG 35

EXPERIMENT 8A
DOG 36

Figure 12. Postexercise blood lactate levels for dog 40.
            Calculated difference between beginning and
            termination of exposure to yttrium oxide is
            +1.0 mg./100 ml.

Figure 13. Postexercise blood lactate levels for dog 41.
            Calculated difference between beginning and
            termination of exposure to yttrium oxide is
            -3.0 mg./100 ml.

Figure 14. Postexercise blood lactate levels for dog 42.
            Calculated difference between beginning and
            termination of exposure to yttrium oxide is
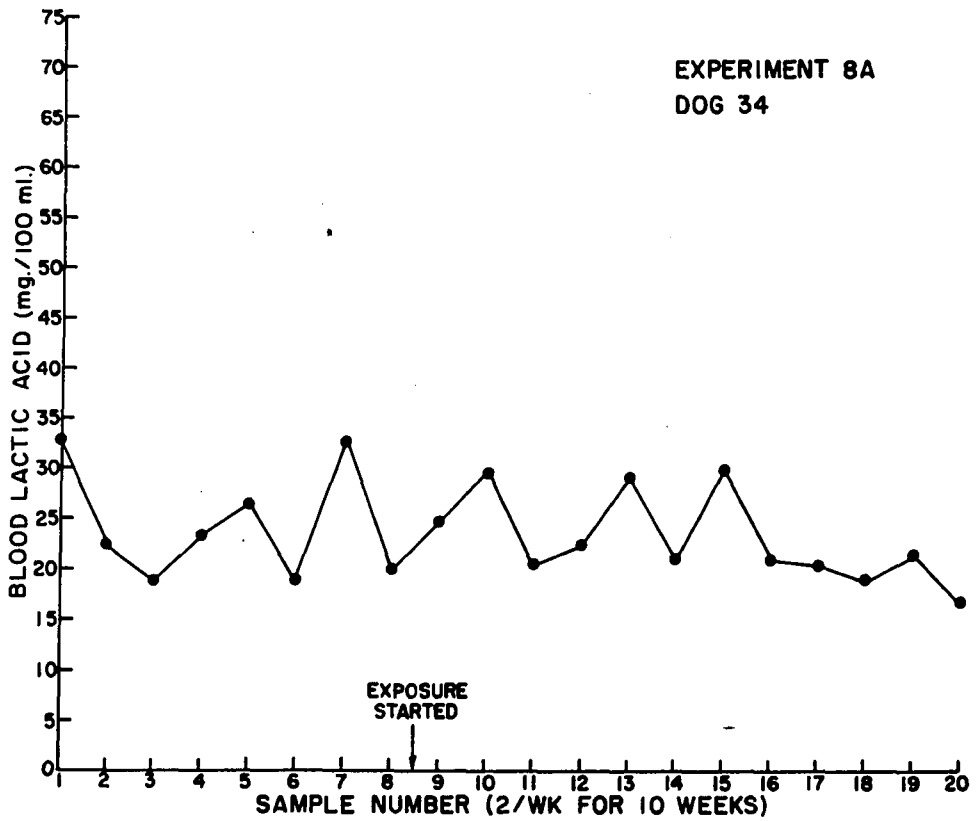   +10.0 mg./100 ml.

Figure 15. Postexercise blood lactate levels for dog 44.
            Calculated difference between beginning and
            termination of exposure to yttrium oxide is
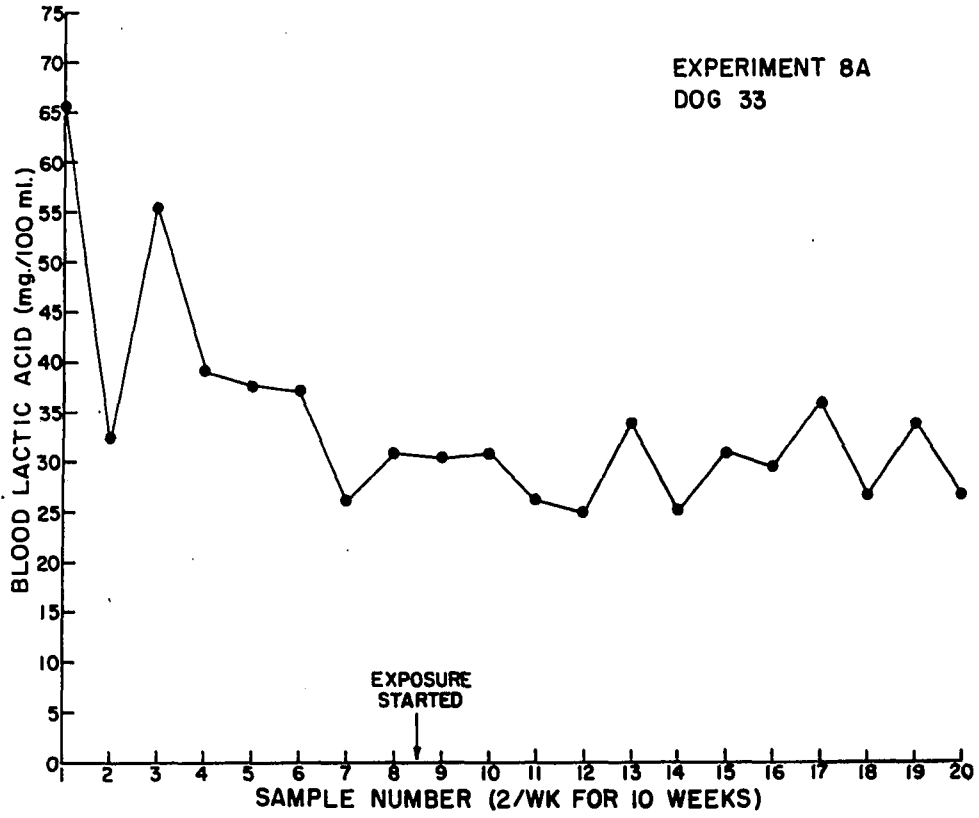   +30.0 mg./100 ml.

EXPERIMENT 8B
DOG 42

EXPERIMENT 8B
DOG 44

Figure 16. Postexercise blood lactate levels for dog 45.
Calculated difference between beginning and
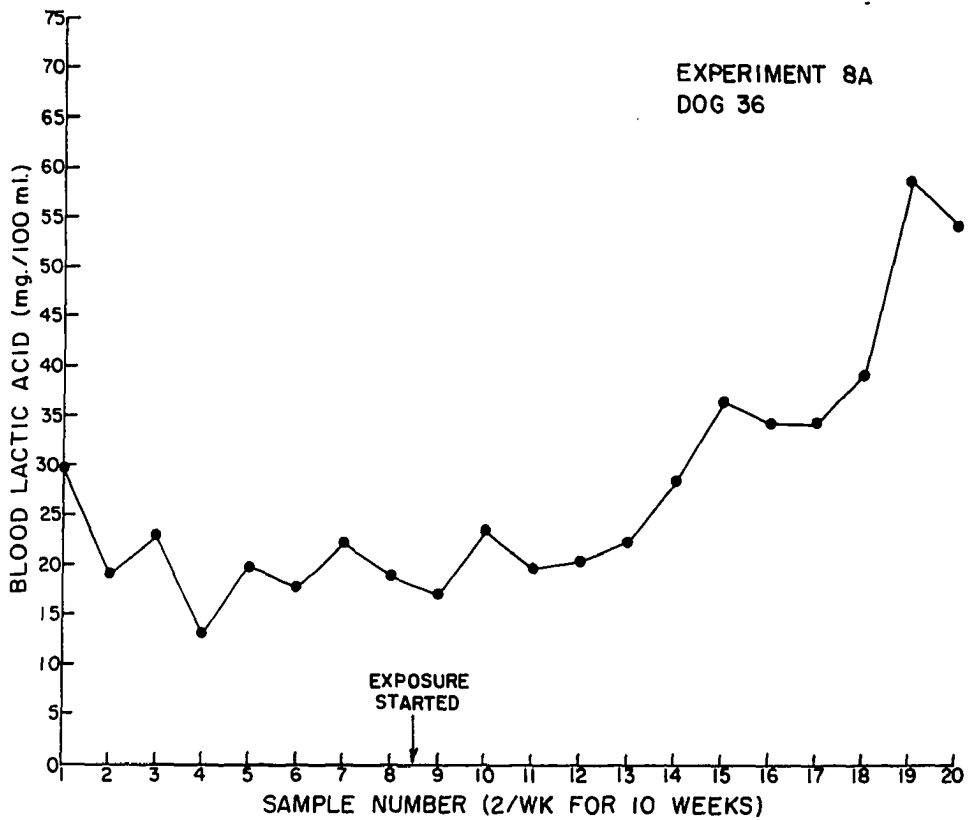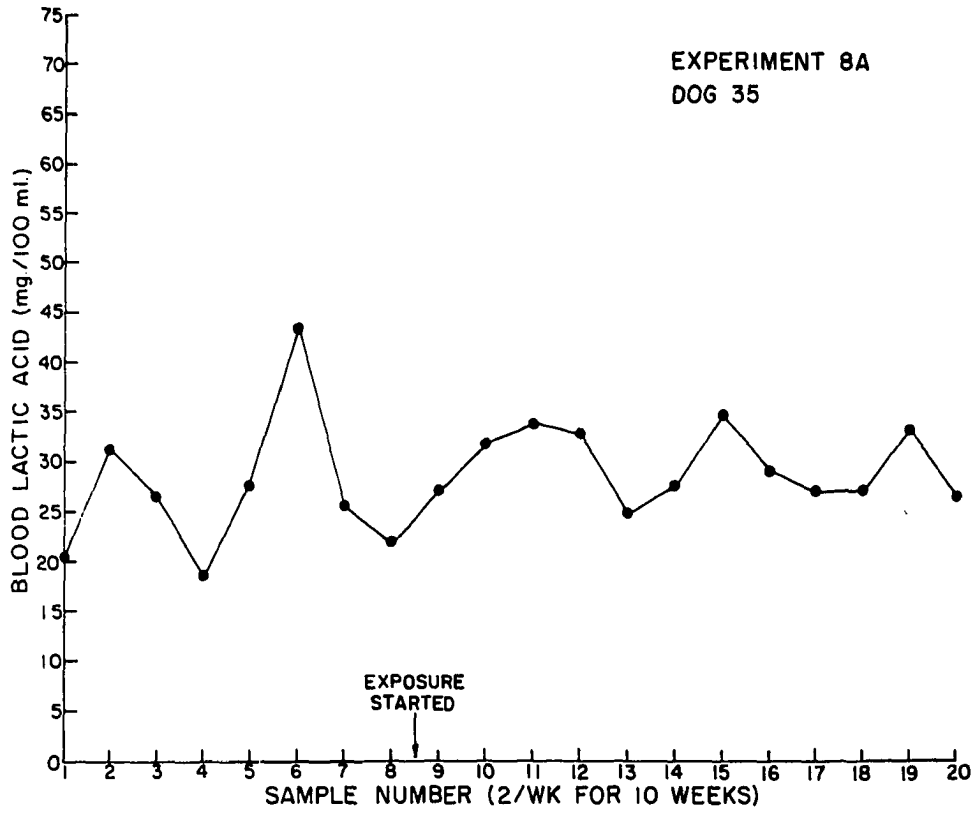termination of exposure to yttrium oxide is
+7.0 mg./100 ml.

Figure 17. Postexercise blood lactate levels for dog 46.
Calculated difference between beginning and
termination of exposure to yttrium oxide is
+1.0 mg./100 ml.

Figure 18. Postexercise blood lactate levels for dog 47.
Calculated difference between beginning and
termination of inhalation exposure to yttrium
oxide is -1.0 mg./100 ml.

Figure 19. Postexercise blood lactate levels for dog 48.
Calculated difference between beginning and
termination of inhalation exposure to yttrium
oxide is 0.0 mg./100 ml.

EXPERIMENT 8C
DOG 47

SAMPLE NUMBER (2/WK FOR 10 WEEKS)

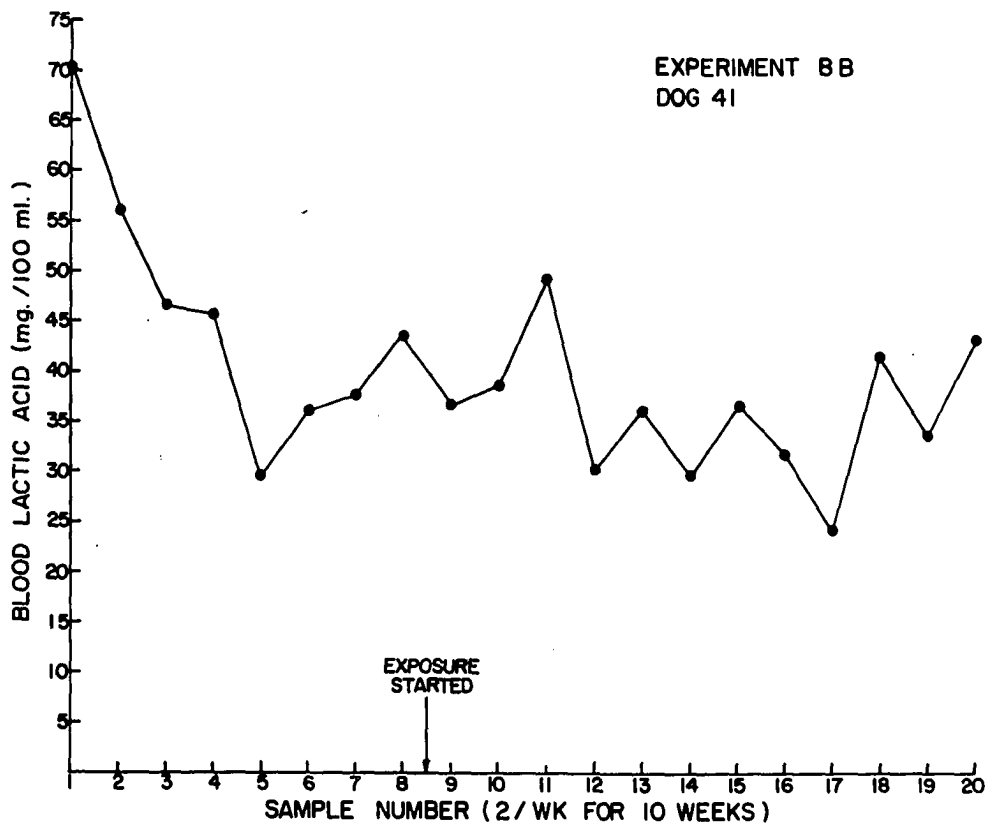EXPERIMENT 8C
DOG 48

SAMPLE NUMBER (2/WK FOR 10 WEEKS)

Figure 20. Postexercise blood lactate levels for dog 49.
Calculated difference between beginning and
termination of inhalation exposure to yttrium
oxide is +22.0 mg./100 ml.

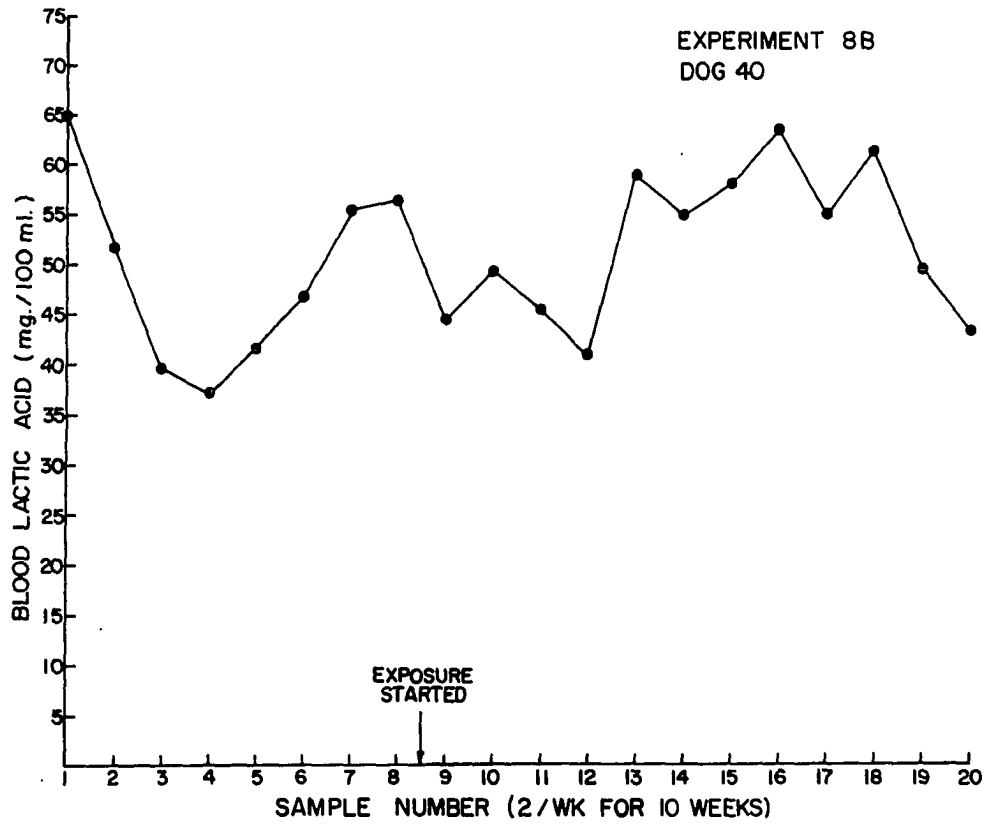Figure 21. Postexercise blood lactate levels for dog 51.
Calculated difference between beginning and
termination of inhalation exposure to yttrium
oxide is +5.0 mg./100 ml.

EXPERIMENT 8C
DOG 49

BLOOD LACTIC ACID (mg./100 ml.)

EXPOSURE STARTED

SAMPLE NUMBER (2/WK FOR 10 WEEKS)

EXPERIMENT 8C
DOG 51

BLOOD LACTIC ACID (mg./100 ml.)

EXPOSURE STARTED

SAMPLE NUMBER (2/WK FOR 10 WEEKS)
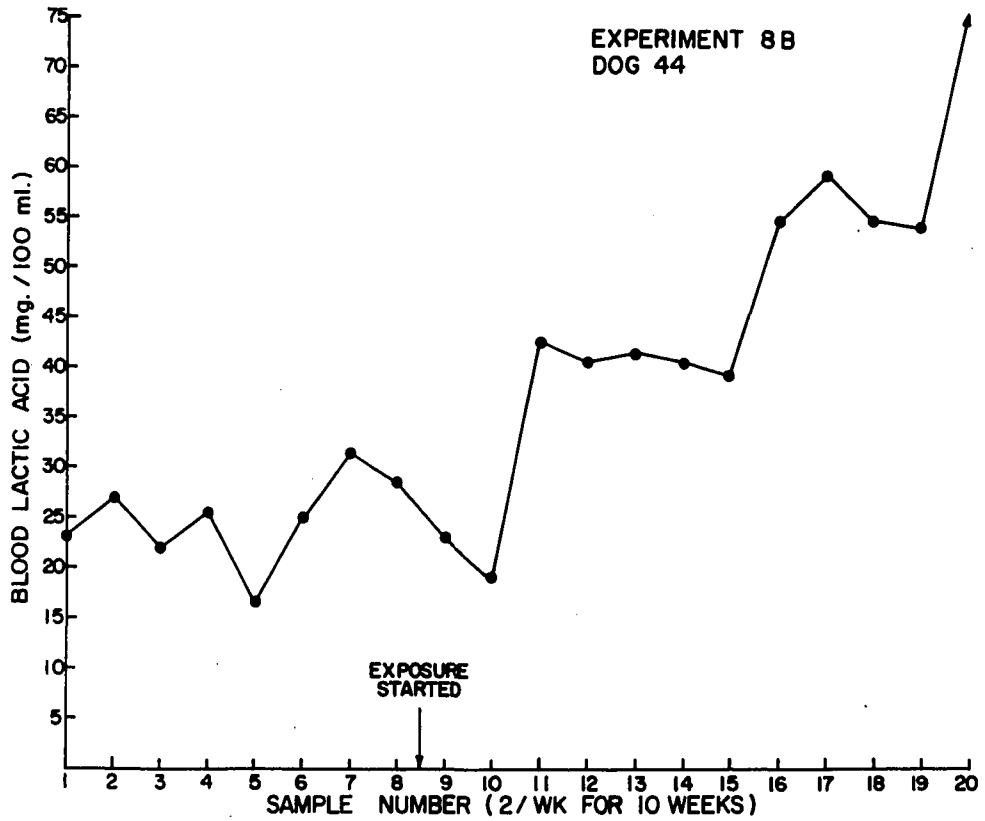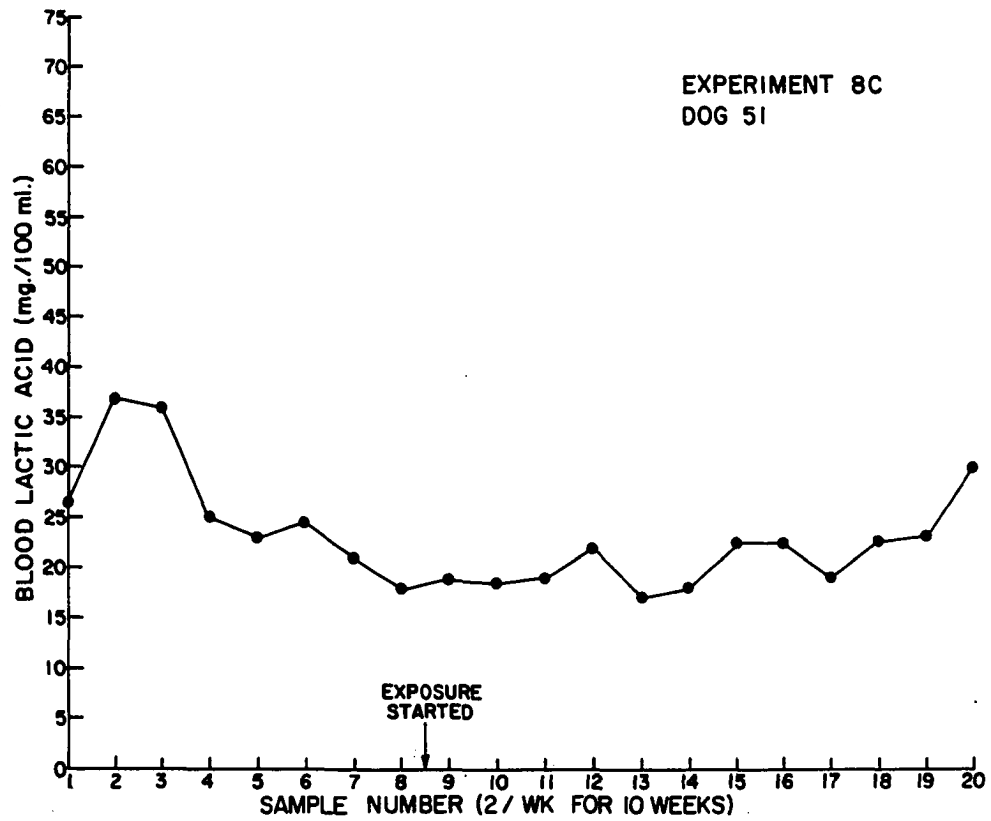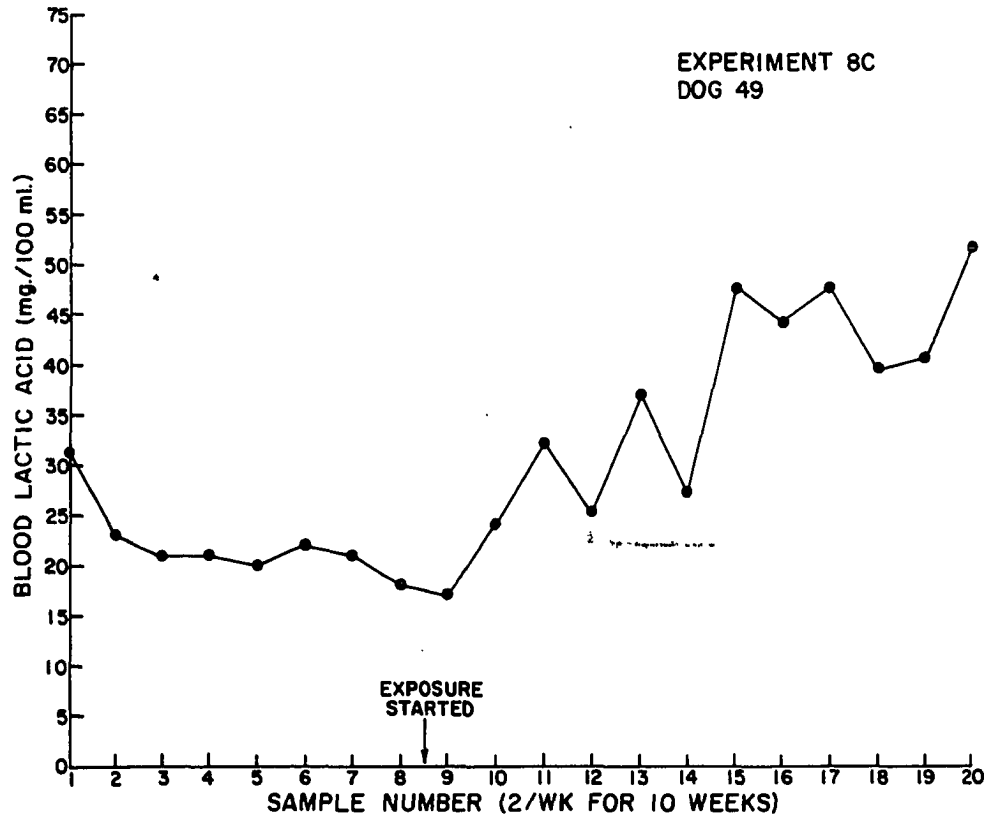
Figure 22.   Postexercise blood lactate levels for dog 53.
             Calculated difference between beginning and
             termination of inhalation exposure to yttrium
             oxide is +1.0 mg./100 ml.

Figure 23.   Postexercise blood lactate levels for dog 54.
             Calculated difference between beginning and
             termination of inhalation exposure to yttrium
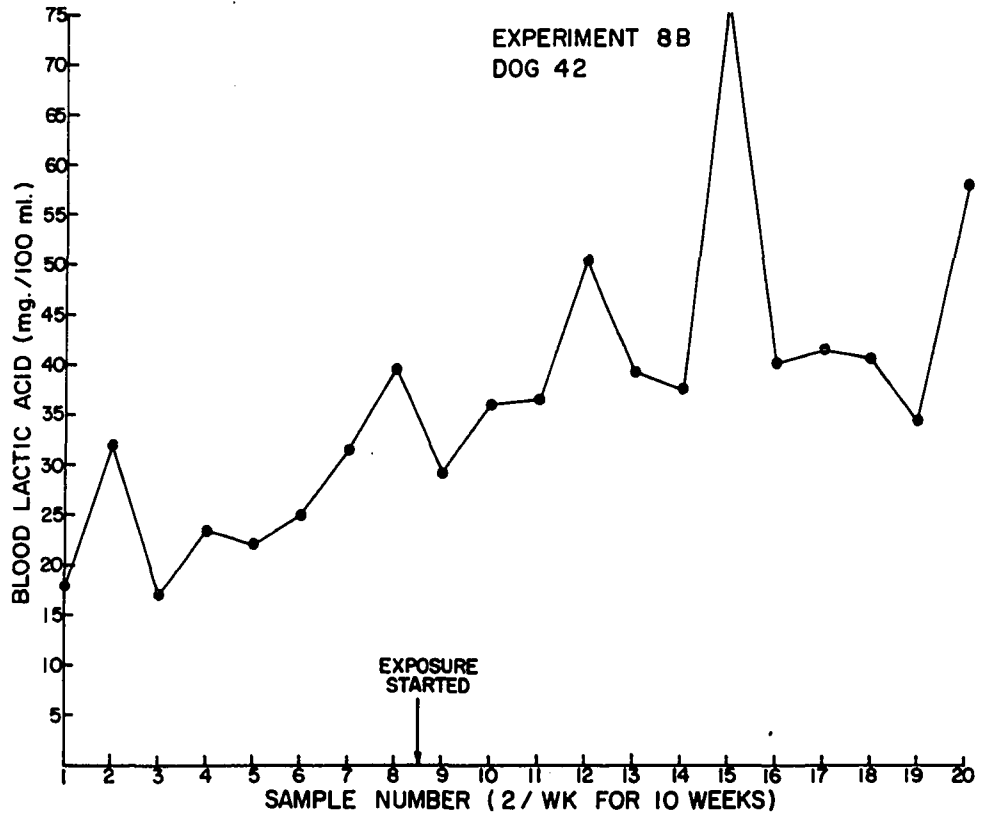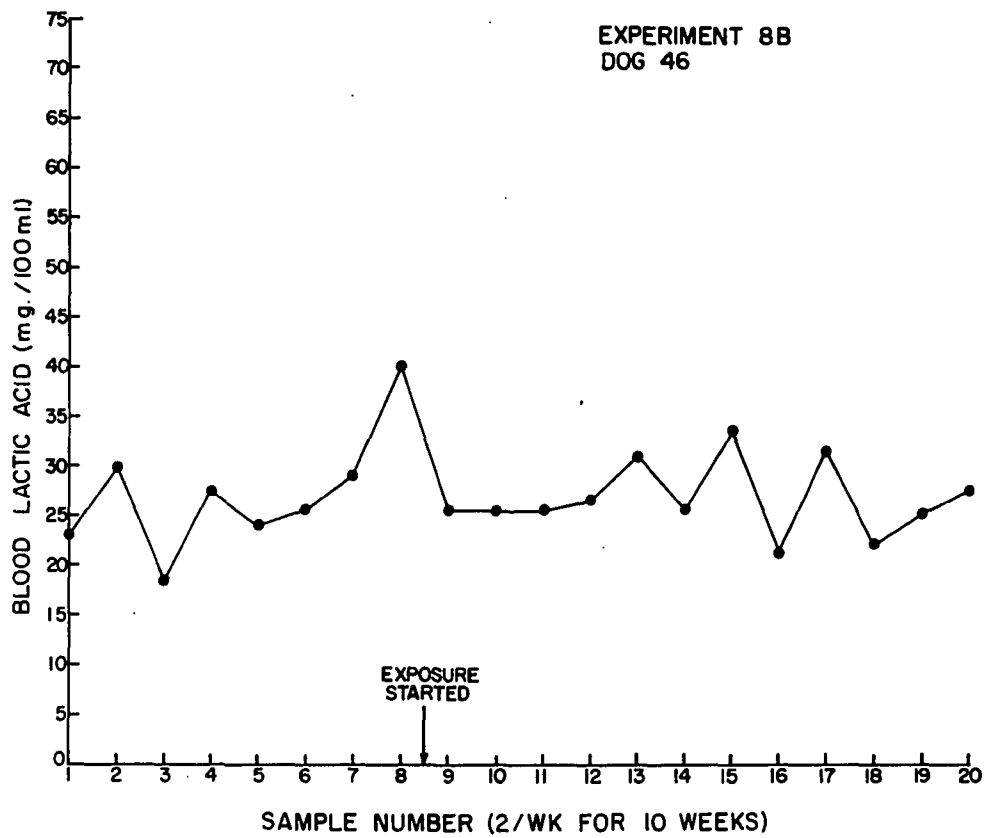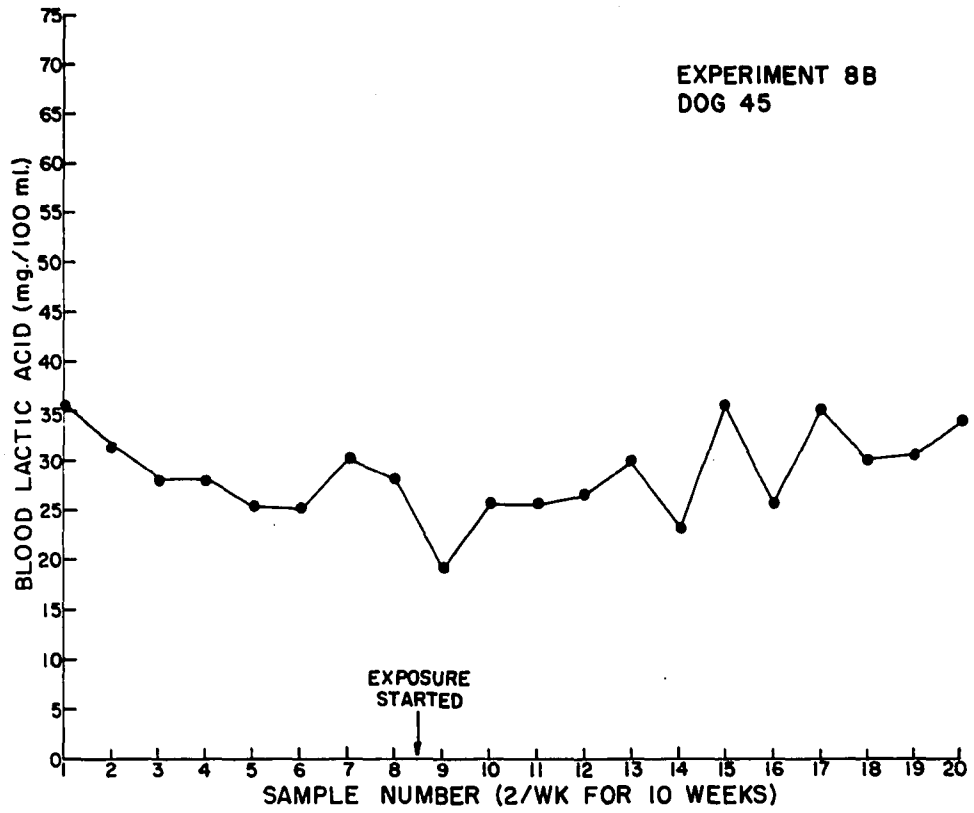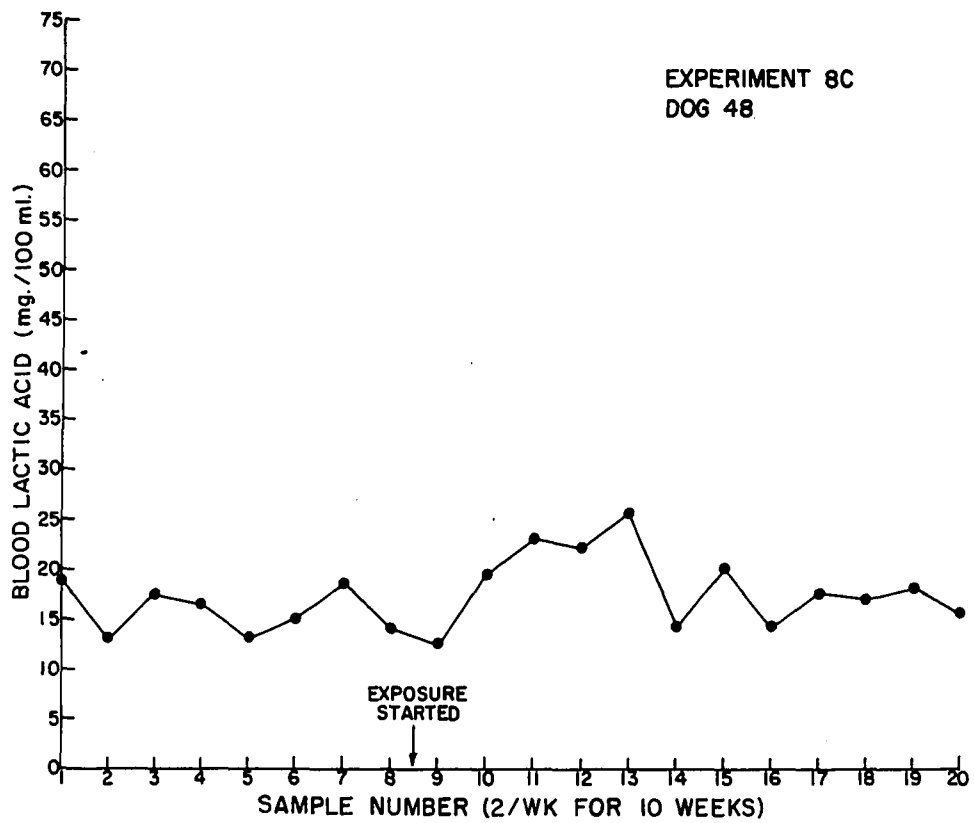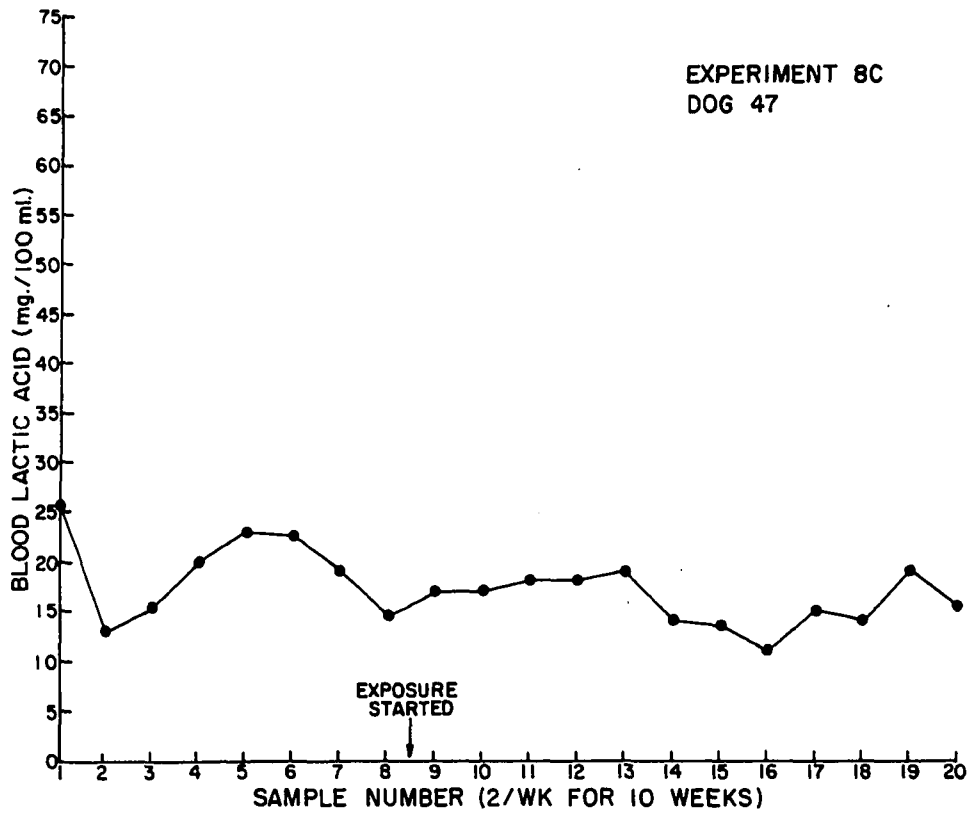   oxide is -3.0 mg./100 ml.

EXPERIMENT 8C
DOG 53

EXPERIMENT 8C
DOG 54
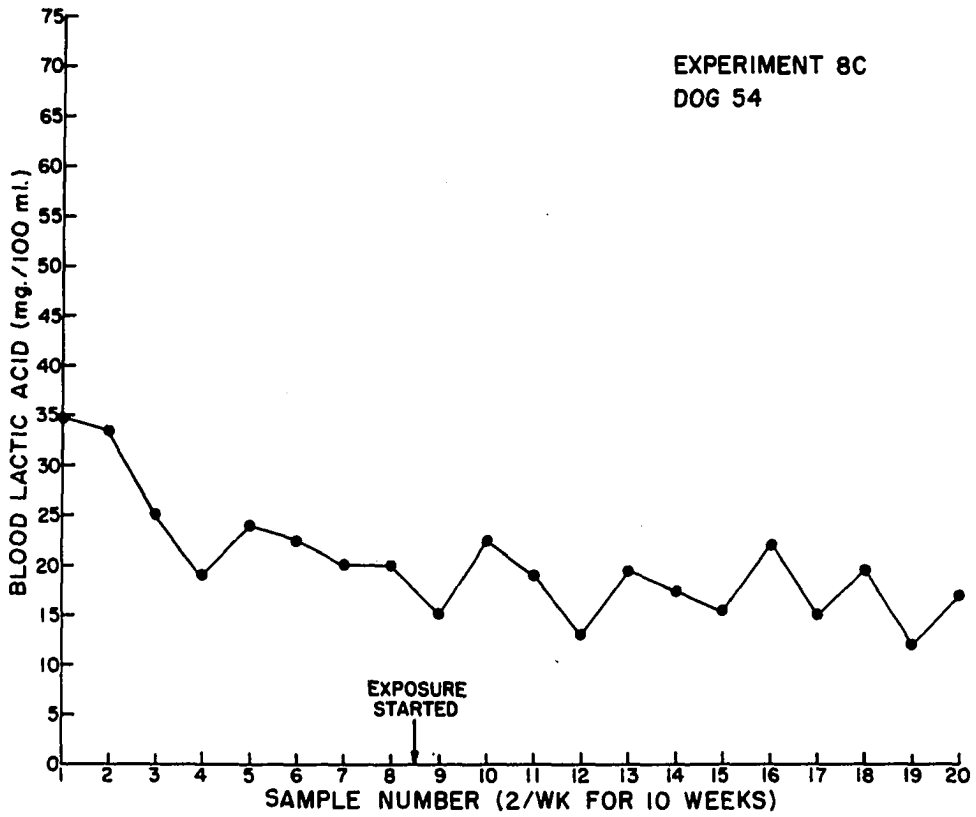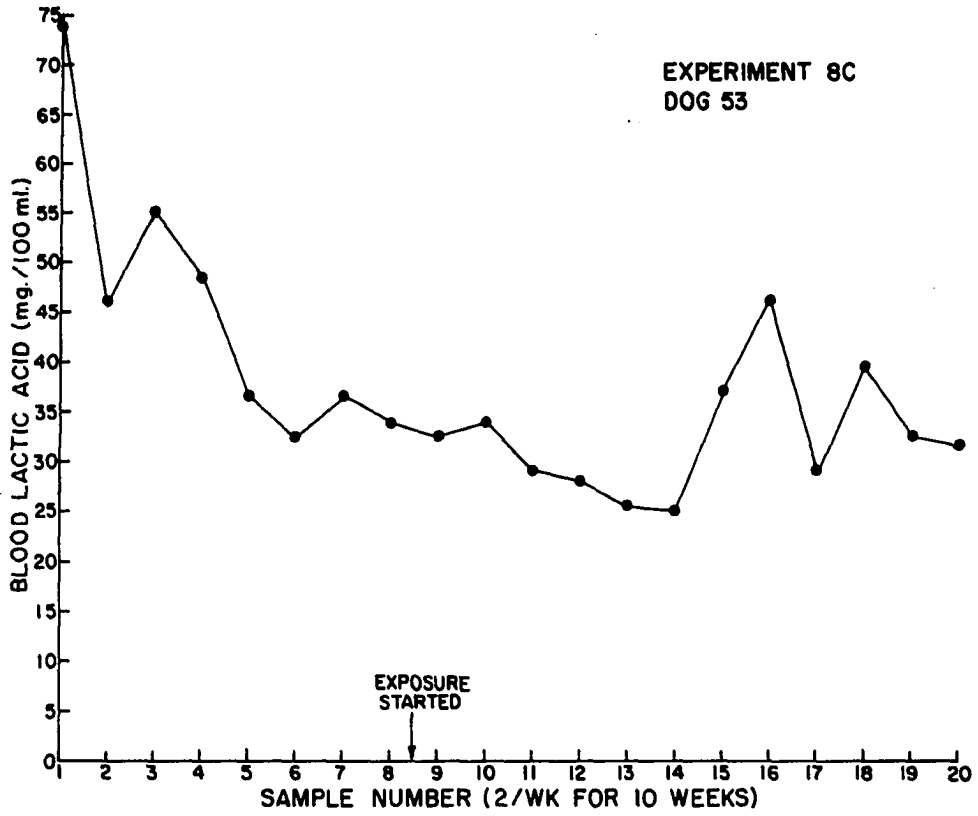
Table 7. Postexercise blood lactate concentration (mg./100 ml.) and differences in dogs at the beginning and termination of 30 exposure days (180 exposure hours) to yttrium oxide

Exp. no.	Dog no.	Sex	Blood lactate concentration (beginning)	(termination)	Differences
8A	31	M	30.0	24.0	-6.0
	32	M	17.0	21.0	+4.0
	33	M	30.0	31.0	+1.0
	34	F	27.0	20.0	-7.0
	35	F	27.0	29.0	+2.0
	36	F	20.0	46.0	+26.0
				Mean	+3.33
8B	40	M	51.0	52.0	+1.0
	41	M	39.0	36.0	-3.0
	45	M	26.0	33.0	+7.0
	42	F	34.0	44.0	+10.0
	44	F	26.0	56.0	+30.0
	46	F	26.0	27.0	+1.0
				Mean	+7.67
8C	47	M	17.0	16.0	-1.0
	48	M	17.0	17.0	0.0
	49	M	23.0	45.0	+22.0
	51	F	19.0	24.0	+5.0
	53	F	32.0	33.0	+1.0
	54	F	19.0	16.0	-3.0
				Mean	+4.00
				Overall mean	+5.00
				Standard deviation	10.60

where: $\bar{d}$ is the mean of the difference.

MS_E is the mean square of the error.

18 represents the number of dogs exposed.

The hypotheses tested were as follows:

$H_o : \mu_D = 0$ Analysis of Difference (D)

$H_A : \mu_D > 0$

Table 8.   Analysis of variance of the differences in postexercise blood lactate as a result of 30 days exposure (180 exposure hours) to yttrium oxide

Source of variation	d.f.	S.S.	M.S.	F
Replication	2	65.33	32.67	0.260
Sex	1	88.89	88.89	0.708
Error	14	1757.78	125.55	
Total	17	1912.00		

It was assumed that with repeated treadmill exercise under conditions of no exposure to yttrium oxide a reduction in postexercise blood lactate might be expected. Thus, the one-tailed t-test seemed justified. The calculated t was 1.894 and thus the null hypothesis was rejected and the alternate hypothesis accepted with significance at the .05 level.

It is observed that a very positive increase occurred in three dogs, one from each experiment. Without doubt, these individuals were responsible for making the overall mean

difference significant. The very positive increases noted in dogs 36, 44, and 49 (Figures 11, 15, and 20), however, indicate that some alteration has definitely occurred in their ability to transfer, transport, or utilize oxygen. It became increasingly more difficult for these dogs to complete their 10-minute exercise period toward the end of the experiment.

It is then logical to consider that perhaps a longer duration of exposure might have caused a considerable increase in postexercise blood lactate in other dogs tested. One also wonders if the exercise imposed upon the individual dogs was sufficient to be reflected by an increase in postexercise blood lactate over and above the compensatory mechanisms of the body. There is no easy formula for predicting the latter possibility. It was pointed out earlier that certain dogs respond to treadmill exercising quite favorably while others are more reluctant to attain increased work output. The ideal situation would be to select only those dogs which responded favorably and subject them to a greater work task. An alternative might be to discover methods of training which would promote a greater percentage of dogs to be good runners on the treadmill. The latter might include the method of puppy husbandry and early training to the treadmill. The concept of a longer exposure to yttrium oxide having an effect on increasing postexercise blood lactate could be accomplished under the conditions of training as used in this experiment. It was assumed that the work output was sufficient to be reflected in a higher postexercise

blood lactate over and above the compensatory mechanisms of the body. This was definitely the case in dogs 36, 44, and 49. It is not known how much longer it would have been necessary to expose the dogs to obtain more individuals with an increased postexercise blood lactate. Toward the end of each of the three experiments it was tempting to speculate on some individuals which appeared to be showing an increased blood lactate concentration (Figures 10, 12, and 14).

The difference in estimated dosage of yttrium oxide did not appear to have any influence on postexercise blood lactate. This was apparent from the results of the analysis of variance. In future experiments of this nature it would be advisable to select dogs which demonstrate an exceptional aptitude for treadmill running and also to consider extending the period of exposure for those dogs which have not shown an increase in blood lactate concentration.

Erythrocyte Volume

The difference in erythrocyte volume for each dog was calculated on the basis of ml./kg. of body weight. The value obtained just prior to exposure was subtracted from the value obtained at the end of the 30-day exposure period. The results obtained from this analysis are presented in Table 9. The overall mean of the difference was -3.9 ml./kg. of body weight. An analysis of variance was performed and is presented in Table 10. The F-test (Snedecor, 1956) was applied and it

Table 9. Erythrocyte volumes (ml./kg. of body weight) and differences in dogs at the beginning and termination of 30 exposure days (180 exposure hours) to yttrium oxide and treadmill exercise

Exp. no.	Dog no.	Sex	Erythrocyte volume (beginning)	(termination)	Differences
8A	31	M	45.5	43.7	-1.8
	32	M	47.5	43.0	-4.5
	33	M	32.6	30.9	-1.7
	34	F	44.1	53.4	+9.3
	35	F	41.2	35.8	-5.4
	36	F	37.4	34.3	-3.1
				Mean	-1.2
8B	40	M	31.4	35.4	+4.0
	41	M	60.8	40.1	-20.7
	45	M	37.8	52.7	+14.9
	42	F	50.8	37.5	-13.3
	44	F	40.6	28.4	-12.2
	46	F	63.0	57.0	-6.0
				Mean	-5.6
8C	47	M	43.1	41.2	-1.9
	48	M	50.9	40.5	-10.4
	49	M	45.1	37.5	-7.6
	51	F	52.2	45.3	-6.9
	53	F	44.3	47.6	+3.3
	54	F	53.7	47.1	-6.6
				Mean	-5.0
				Overall Mean	-3.9
				Standard Deviation	8.3

indicated that there was no significant difference due to replications and sex. The test of overall significance of the mean was tested with Student's t-test (Snedecor, 1956), again using the mean square of error and 14 degrees of freedom. The following hypotheses were tested:

$$H_o: \mu_D = 0$$

$$H_A: \mu_D \neq 0$$

The calculated t was 1.867 and this was found to be significant at the 0.10 level. It is noted, therefore, that the mean of the difference was not highly significant.

Table 10. Analysis of variance of the differences in erythrocyte volume as a result of 30 days exposure (180 exposure hours) to yttrium oxide and treadmill exercise

Source of variation	d.f.	S.S.	M.S.	F
Replication	2	67.6	33.8	0.430
Sex	1	7.0	7.0	0.089
Error	14	1100.0	78.6	
Total	17	1174.6		

It was interesting to note that the mean of the difference was negative. One might assume that with increased tissue demands for oxygen an increase in erythrocyte volume might occur. Could it be that enough yttrium oxide was being transported

from the lungs to other tissues to have some inhibiting effect upon erythropoiesis? If such an effect were taking place, it might never have been known unless the test experiment involved exercise, such as this, and imposed upon the tissues a positive need for oxygen. Here again, one might have observed a more significant decrease in erythrocyte volume if the period of exposure and exercise had been extended.

It was interesting to note that the dog which displayed the greatest increase in postexercise blood lactate (Dog No. 44) also had the lowest erythrocyte volume. It decreased from 40.6 ml./kg. of body weight just prior to exposure to 28.4 ml./ kg. of body weight at the end of exposure. Dogs 36 and 49 also had erythrocyte volumes of 34.3 and 37.5 ml./kg. of body weight, respectively, at the termination of exposure and showed marked increases in postexercise lactate. Four other dogs had an erythrocyte volume less than 40 ml./kg. of body weight and three of these (Dogs 35, 40, and 42) were dogs in which it appeared that a marked increase in lactate would soon occur (Figures 10, 12, and 14).

The loss of injected isotope (Cr^{51}) observed during the postexposure erythrocyte volume determination in Experiment 8C was a fortunate observation. Had it not been observed, the low count obtained would have been calculated as an increase in erythrocyte volume. It was immediately supposed that the same phenomenon had occurred previously and had gone undetected. However in looking over the results, it can be seen that the

values are in general quite consistent. There are a few values which appear somewhat high and perhaps agglutination to a minor factor did occur with subsequent loss of count. It is realized that the use of autologous blood involves considerably more time for preparation. Unless facilities are available for careful agglutination testing, it would be recommended that future erythrocyte volume determinations in dogs with Cr^{51} be done with autologous blood.

Inasmuch as our histologic control dogs were not consistent runners, no real indication was obtained as to whether or not the erythrocyte volume increased or decreased as a result of exercise without exposure.

Plasma Volume

The difference in plasma volume calculated for each dog was also expressed as ml./kg. of body weight. The value obtained prior to exposure was subtracted from the value obtained after 30 days exposure (180 exposure hours) and noted as the difference. The results obtained from this analysis are shown in Table 11. The overall mean of the differences was -0.4 ml./ kg. of body weight. An analysis of variance was performed and is presented in Table 12. The F-test (Snedecor, 1956) was applied and a significant difference was detected due to replications but no significant difference due to sex. The overall significance of the mean was tested with Student's t-test (Snedecor, 1956) using the mean square of error and 14

Table 11. Plasma volumes (ml./kg. of body weight) and differences in dogs at the beginning and termination of 30 exposure days (180 exposure hours) to yttrium oxide and treadmill exercise

Exp. no.	Dog no.	Sex	Plasma volume (beginning)	Plasma volume (termination)	Differences
8A	31	M	523.4	590.6	+5.7
	32	M	467.6	504.4	+0.3
	33	M	614.0	629.8	+2.6
	34	F	484.7	545.6	+4.6
	35	F	362.4	376.5	-3.1
	36	F	405.9	442.8	+0.8
				Mean	+1.8
8B	40	M	421.4	422.3	-0.3
	41	M	420.5	345.4	-7.3
	45	M	655.9	596.5	-5.6
	42	F	593.2	567.5	-2.6
	44	F	521.6	492.8	-2.8
	46	F	590.3	526.4	-7.0
				Mean	-4.3
8C	47	M	763.0	792.0	-0.6
	48	M	481.0	509.0	+2.0
	49	M	428.0	480.0	+5.3
	51	F	442.0	464.0	+0.8
	53	F	396.0	397.0	+1.6
	54	F	563.0	557.0	-1.0
				Mean	+1.4
				Overall Mean	-0.4
				Standard Deviation	3.86

degrees of freedom.

The following hypotheses were tested:

$$H_o : \mu_D = 0$$

$$H_A : \mu_D \neq 0$$

The calculated t was 0.606. The null hypothesis was thus accepted and the overall mean of the difference was not considered significant.

Table 12. Analysis of variance of the difference in plasma volume as a result of 30 days exposure (180 exposure hours) to yttrium oxide and treadmill exercise

Source of variation	d.f.	S.S.	M.S.	F
Replication	2	137.58	68.79	8.819[a]
Sex	1	6.48	6.48	0.831
Error	14	109.26	7.80	
Total	17	253.32		

[a]$p < 0.01$.

It was expected that the mean of the difference of plasma volume would be positive since the mean of the difference of the erythrocyte volume was negative. This would represent an attempt of the body to maintain the blood volume at a constant level (Guyton, 1961). This was the case in Experiments 8A and 8C. In Experiment 8B the mean of the difference was -4.3 ml./kg. of body weight. This finding in conjunction with the mean

decrease in erythrocyte volume of Experiment 8B represents a slight decrease in vascular capacity. Guyton (1961) states that the inverse relationship between cell and plasma volume is not entirely volume for volume because changes in red cell concentration change the viscosity of the blood. The viscosity in turn can exert pressure effects that alter the vascular capacity. In anemia it is noticed that the blood volume is usually slightly decreased. The greatest reduction in erythrocyte volume occurred in Experiment 8B, and this might explain the significant difference among replications as noted in the analysis of variance for plasma volumes.

Davis and Brewer (1935) reported on an experiment wherein four dogs were exercised, two by swimming (two hours daily) and two by treadmill (25% grade, six miles daily, speed not given). They found that the blood volume was markedly decreased in the first week of exercise in three of the four dogs. However, after six weeks of exercise, all dogs showed a blood volume higher than the pre-exercise level. It was apparent that no increase in blood volume occurred in this experiment with yttrium oxide which further suggested that perhaps erythropoiesis had been inhibited.

Hemoglobin

The differences in hemoglobin concentration between the beginning and termination of exposure are expressed as grams per 100 ml. of blood. The analysis of the difference is

presented in Table 13. The mean of the difference is +0.35 gms./100 ml. of blood. The analysis of variance for the differences in hemoglobin concentrations is presented in Table 14. The F-test (Snedecor, 1956) was applied and it was noted that there was no significant difference due to replications and sex. The application of Student's t-test (Snedecor, 1956) to the overall mean of the difference indicated that the positive increase noted was not significant. The calculated t was 1.094 which was less than a t of 2.145 needed for significance at the 0.05 level.

It is worthy to note that the dogs which had a large increase in postexercise blood lactate and a corresponding decrease in erythrocyte volume (Dogs 36, 44, and 49), presented a decrease in hemoglobin concentration. In Dog Number 42, where it appeared that a marked increase in lactate might soon occur, a low hemoglobin concentration was apparent at the beginning of exposure (11.5 gms./100 ml.). No further reduction occurred, however, and a rise in lactate was noted at the end of the experiment (+10 mg./100 ml.).

Leukocytes

The differences noted in the white blood cell counts between the beginning and termination of the exposure are recorded in Table 15. The mean of the difference is +2990 leukocytes per cu. mm. of blood. An analysis of variance was performed and is presented in Table 16. After applying the F-test

Table 13. Hemoglobin concentrations (gms./100 ml. blood) and differences in dogs at the beginning and termination of 30 exposure days (180 exposure hours) to yttrium oxide and treadmill exercise

Exp. no.	Dog no.	Sex	Hemoglobin concentrations (beginning)	(termination)	Differences
8A	31	M	16.00	17.50	+1.5
	32	M	15.00	15.00	0.0
	33	M	12.00	12.00	0.0
	34	F	13.50	16.00	+2.5
	35	F	12.50	15.00	+2.5
	36	F	14.00	13.00	-1.00
				Mean	+0.92
8B	40	M	14.5	15.25	+0.75
	41	M	15.25	16.75	+1.50
	45	M	14.50	16.00	+1.50
	42	F	11.50	11.50	0.00
	44	F	15.25	13.50	-1.75
	46	F	15.25	16.50	+1.25
				Mean	+0.54
8C	47	M	16.50	15.50	-1.00
	48	M	19.50	17.00	-2.50
	49	M	18.25	17.00	-1.25
	51	F	16.50	17.50	+1.00
	53	F	17.50	18.75	+0.75
	54	F	16.25	16.75	+0.50
				Mean	-0.42
				Overall Mean	+0.35
				Standard Deviation	1.41

Table 14. Analysis of variance for the difference in hemo-
globin concentrations as a result of 30 days expo-
sure (180 exposure hours) to yttrium oxide and
treadmill exercise

Source of variation	d.f.	S.S.	M.S.	F
Replication	2	5.67	2.84	1.488
Sex	1	1.53	1.53	0.803
Error	14	26.69	1.91	
Total	17	33.89	1.99	

Table 15. Total leukocyte counts (cells/cu. mm. of blood) and differences in dogs at the beginning and termination of 30 exposure days (180 exposure hours) to yttrium oxide and treadmill exercise

| Exp. no. | Dog no. | Sex | Leukocyte count | | Differences |
			(beginning)	(termination)	
8A	31	M	14,300	16,550	+2250
	32	M	11,650	14,250	+2600
	33	M	15,450	19,150	+3700
	34	F	10,600	16,050	+5450
	35	F	11,800	13,750	+1950
	36	F	16,950	15,200	-1750
				Mean	+2367
8B	40	M	10,700	12,800	+2100
	41	M	10,950	13,600	+2650
	45	M	16,900	11,000	-5900
	42	F	11,850	14,600	+2750
	44	F	8,550	14,250	+5700
	46	F	8,950	9,550	+600
				Mean	+1317
8C	47	M	12,900	20,550	+7650
	48	M	10,700	14,750	+4050
	49	M	10,150	13,900	+3750
	51	F	9,050	11,200	+2150
	53	F	7,300	6,950	-350
	54	F	7,600	10,350	+2750
				Mean	+3333
				Overall Mean	+2333
				Standard Deviation	2990

(Snedecor, 1956) it was noted that there was no significant difference due to replications and sex. Application of Student's t-test (Snedecor, 1956), however, to the overall mean of the differences indicates that the increase noted was significant. The mean square of error was used for this test where there were 14 degrees of freedom. The calculated t was 3.144 which was significant at the 0.01 level.

Table 16. Analysis of variance of the differences in total leukocyte counts as a result of 30 days exposure (180 exposure hours) to yttrium oxide and treadmill exercise

Source of variation	d.f.	S.S.	M.S.	F
Replication	2	12.21	6.10	0.616
Sex	1	0.72	0.72	0.072
Error	14	138.64	9.90	
Total	17	151.56		

The increase in white cells which was found to be significant indicates an active response of the body to remove foreign material. Davison (1963) found no significant difference in white cell counts of mice and guinea pigs exposed to inhaled neodymium oxide and their controls. The increase noted here could be a peculiarity of species response, the effects of yttrium oxide, or any of the above combined with treadmill exercise.

Necropsy

A necropsy examination was performed on all dogs immediately following euthanasia with pentobarbital anesthesia and subsequent exsanguination. The lungs of the dogs which had been exposed to yttrium oxide did not have the pink color present as in the dogs used as histologic controls. They presented a reddish gray appearance and were firmer than the controls. The bronchial lymph nodes in the exposed dogs were also enlarged. The lymph nodes were not weighed but they were measured and were found to be 2-3 times longer in length and width. Other body lymph nodes were normal in size. Hyperemia was present in the liver and spleen of all dogs and was attributed to the pentobarbital anesthesia. With regard to gross appearance, all other organs were normal.

Histologic Examination

Infusion of the lungs with 10% formalin via the trachea accomplished the purpose of fixation in order to see more distended alveoli. It is believed that this technique gave a better perspective with regard to determining alveolar wall thickening (Figures 24 and 25). It appears that infusion washed out many macrophages which were in the alveoli and perhaps tore some alveolar cells loose which were about ready to desquamate. Slides prepared by both fixation techniques (placed in 10% formalin and also as described above) were used in their proper perspective to obtain photomicrographs for

Figure 24.   Lung from a control dog which was not infused
              with 10% formalin. Note atelectic alveoli.
              x 156. H. and E. stain.

Figure 25.   Lung from a dog exposed to yttrium oxide for 30
              days and which was infused with 10% formalin.
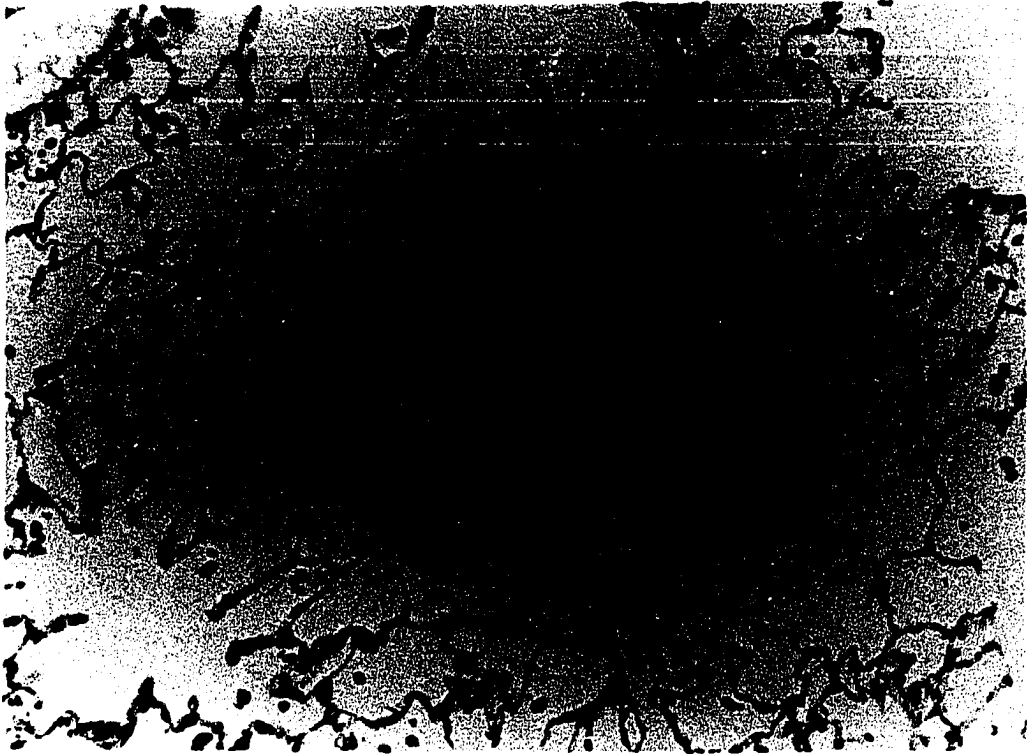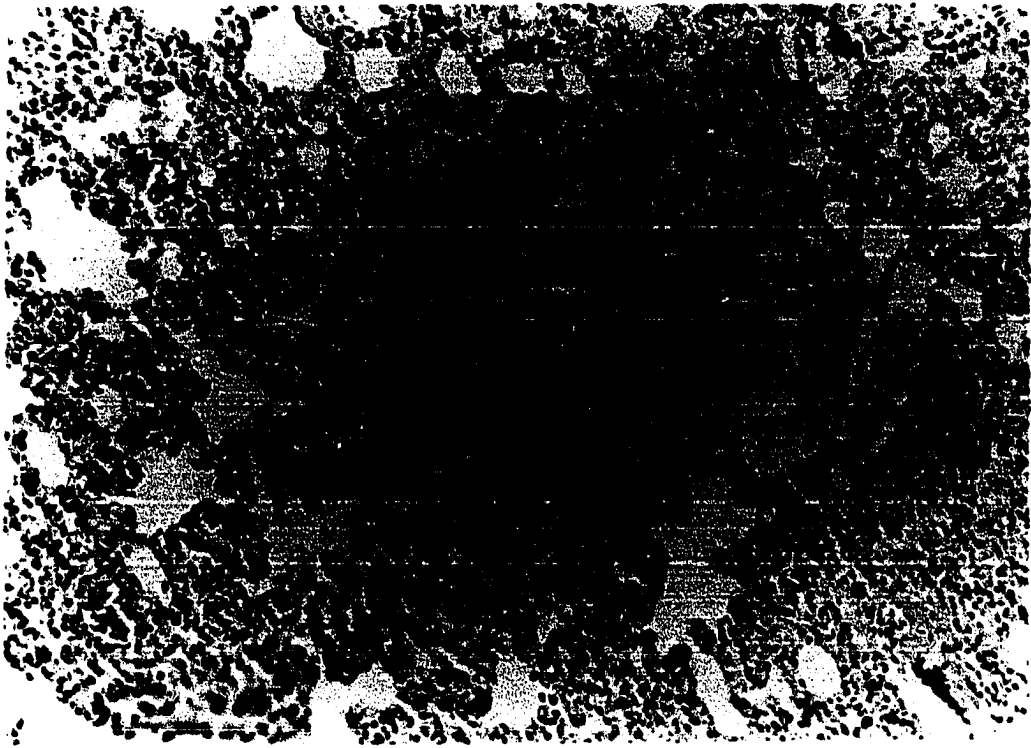   Note distention of alveoli. x 156. H. and E.
              stain.

illustration.

Sections from control and exposed lungs are shown in Figures 26 and 27. Many cellular elements are noted within the alveoli of the exposed lungs. The most apparent histologic change as a result of 30 days exposure (180 exposure hours) to yttrium oxide was the presence of numerous macrophages in the alveoli (Figures 28 and 29). According to Hatch and Gross (1964) these alveolar macrophages or "dust cells" are desquamated alveolar cells. The similarity between the alveolar macrophages and attached, rounded alveolar cells can also be noted. The process whereby the alveolar epithelial cells become alveolar macrophages involves hypertrophy and hyperplasia of intact cells. This process in itself would thus contribute to increased thickness of the pulmonary membrane (Figure 30). The extreme hypertrophy of alveolar epithelial cells prior to desquamation is shown in Figures 31 and 32.

Many leukocytes were also noted in the lung (Figure 33). They were primarily neutrophils. Many presented elongated nuclei and were probably in the process of diapedesis (Figure 34). It was apparent that some leukotactic substance was attracting many leukocytes to the lung and this was reflected by the increased leukocyte count previously noted.

Gomori's one step trichrome method of staining was applied to several slides of lung tissue in an attempt to detect fibrosis. No connective tissue increase was noted.

Hypertrophy of the smooth muscle encircling the bronchioles

Figure 26. Lung from a control dog which was infused with 10% formalin. x 100. H. and E. stain.

Figure 27. Lung from a dog exposed to yttrium oxide for 30 days and which was infused with 10% formalin. Note the cellular elements within the alveoli. x 100. H. and E. stain.
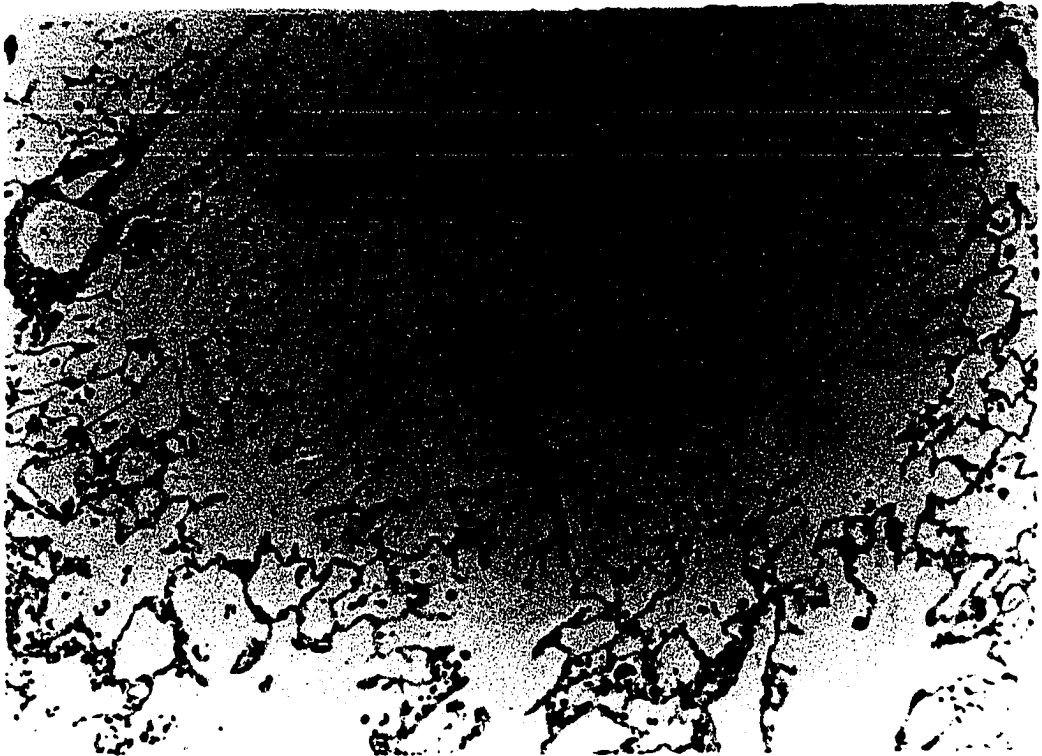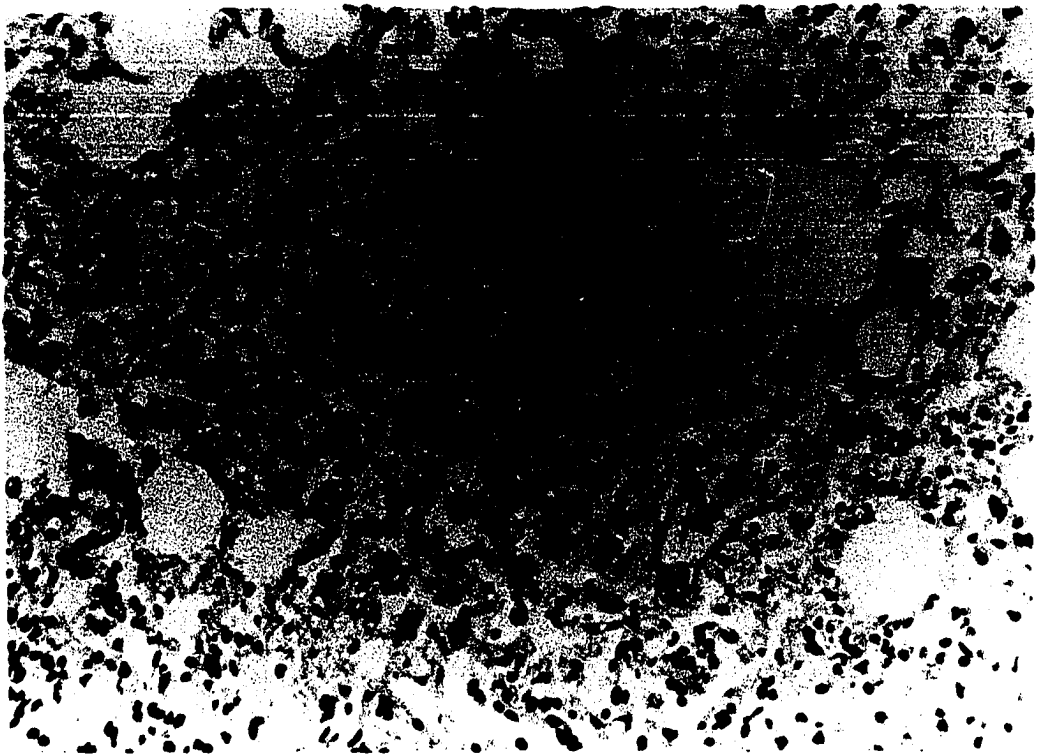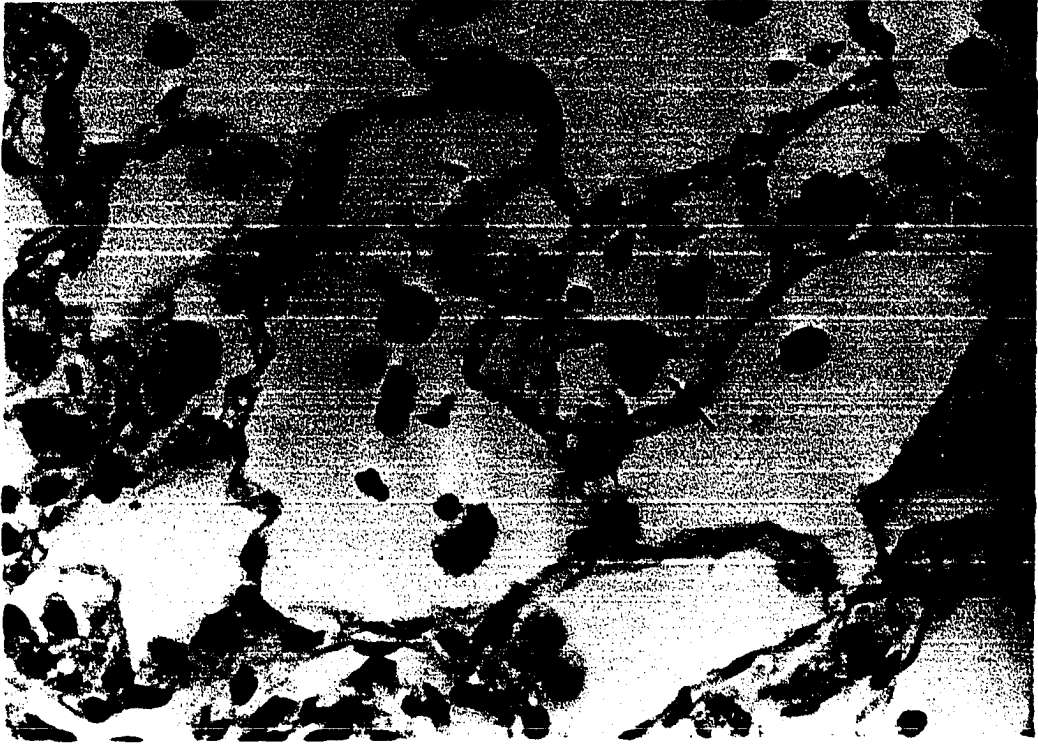
Figure 28.   Lung from a dog exposed to yttrium oxide for 30
             days and which was infused with 10% formalin.
             Arrows point to a free macrophage and an attached,
             rounded, alveolar epithelial cell. Note the
             similarity in morphology. x 624. H. and E. stain.

Figure 29.   Lung from a dog exposed to yttrium oxide for 30
             days and which was not infused with 10% formalin.
             Arrows point to some of the macrophages. x 250.
             H. and E. stain.

Figure 30. Lung from a dog exposed to yttrium oxide for 30 days and which was infused with 10% formalin. Arrows point to alveolar epithelial cell hypertrophy which contributes to increased thickness of the pulmonary membrane. x 624. H. and E. stain.

Figure 31. Lung from a dog exposed to yttrium oxide for 30 days and which was infused with 10% formalin. Arrows point to cells which have undergone extreme hypertrophy prior to desquamation. x 624. H. and E. stain.
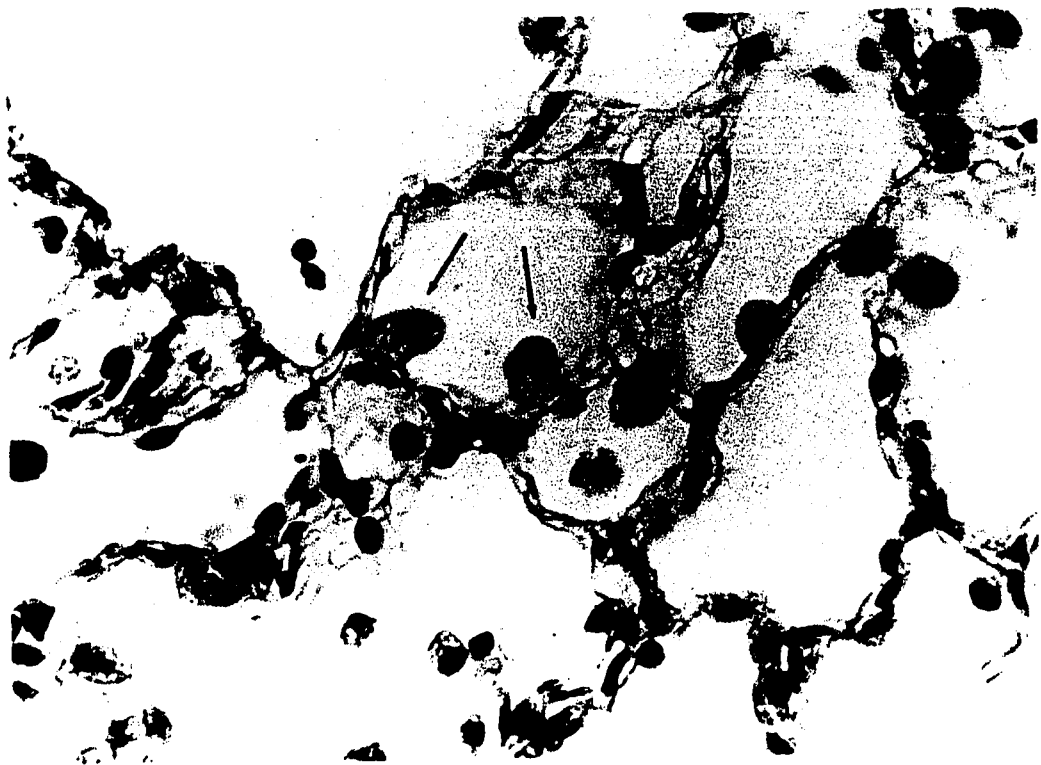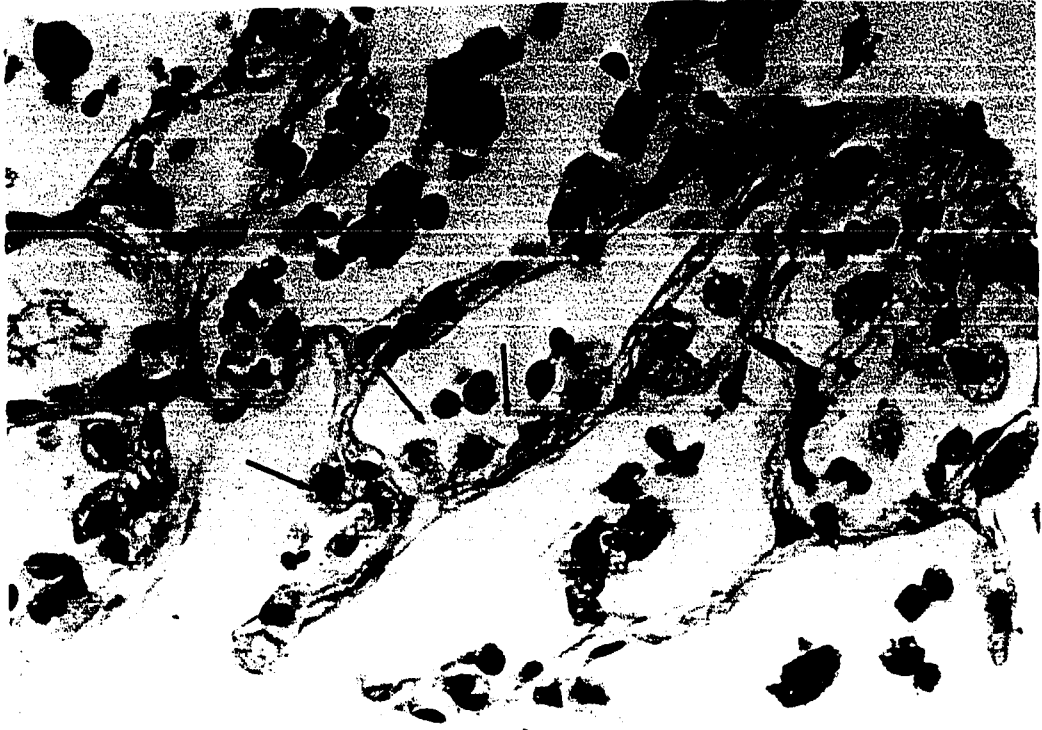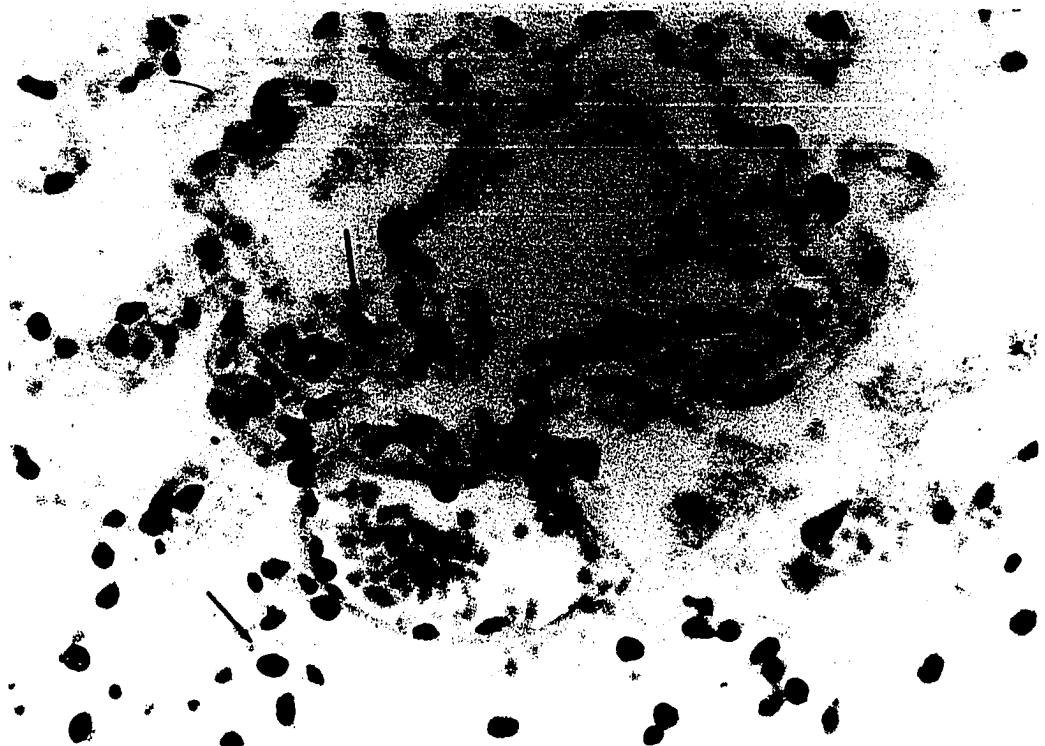
Figure 32. Higher magnification of lung shown in Figure 31. Arrows point to cells which have undergone hypertrophy. x 1560. H. and E. stain.

Figure 33. Lung from a dog exposed to yttrium oxide for 30 days and which was not infused with 10% formalin. Arrows point to some of the many neutrophils. x 624. H. and E. stain.

was noted in the exposed and nonexposed dogs (Figure 35). It is supposed that the treadmill exercise contributed to this.

Examination of the bronchial lymph nodes from the exposed dogs showed the presence of numerous dust-laden macrophages (Figures 36 and 37). It was interesting to note their presence after 30 exposure days. Davison (1963) noted their presence in the tracheo-bronchial lymph nodes of mice after 100 days of inhalation exposure to neodymium oxide. Further, the presence of dust-laden macrophages was noted in guinea pigs after 50 days of inhalation exposure to neodymium oxide. This experiment with dogs does not demonstrate how much sooner than 30 days that the yttrium oxide might have appeared in the bronchial lymph nodes. It does demonstrate that the lymphatics of the dog appear to be quite efficient in the removal of the contaminant from the lung to the bronchial lymph nodes.

Parts of bronchial lymph nodes from three exposed dogs and one control dog were digested in hot dilute nitric acid (1:1). The spectrophotometric procedure as described for the determination of chamber aerosol concentration was then performed. This established that yttrium oxide was in fact present within the bronchial lymph nodes and substantiates the observation of supposed dust particles within the macrophages. An approximate concentration of 0.95 mg./gm. of lymph node (wet basis) was found in the exposed dogs whereas the control dogs were negative.

Histologic examination of heart, liver, spleen,

Figure 34.   Lung from a dog exposed to yttrium oxide for 30
             days and which was not infused with 10% formalin.
             Arrow points to a leukocyte undergoing diapedesis.
             x 2496.   H. and E. stain.

Figure 35.   Lung from a dog exposed to yttrium oxide for 30
             days.   Arrow points to region of peribronchiolar
             smooth muscle hypertrophy.   Hypertrophy present
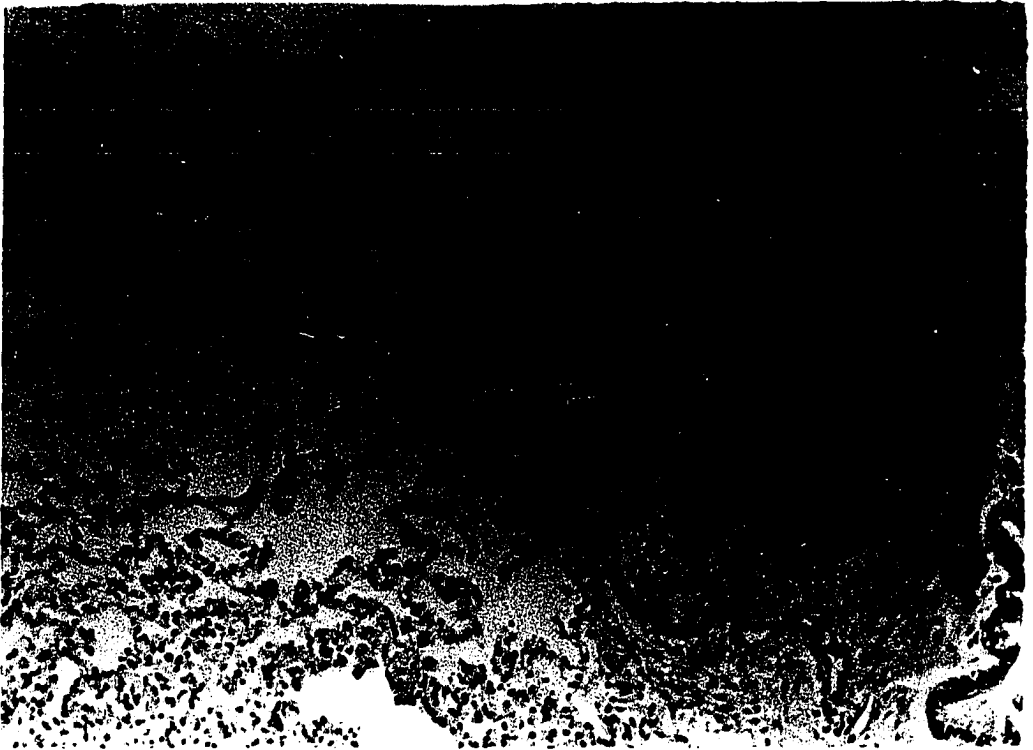   in control dogs also and believed due to treadmill
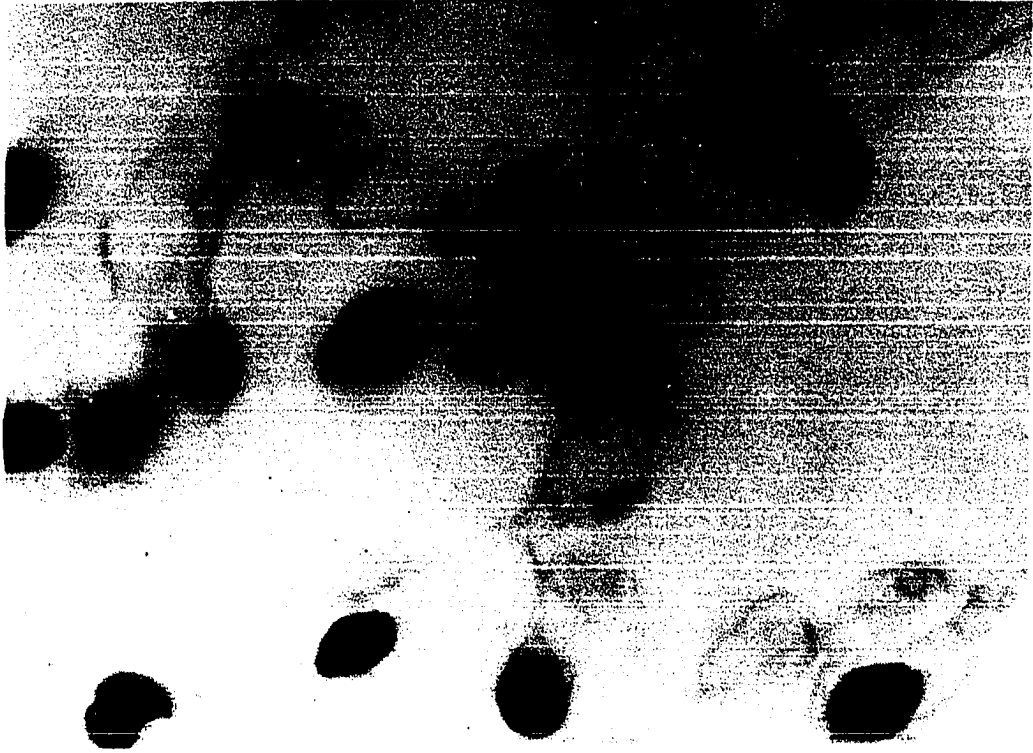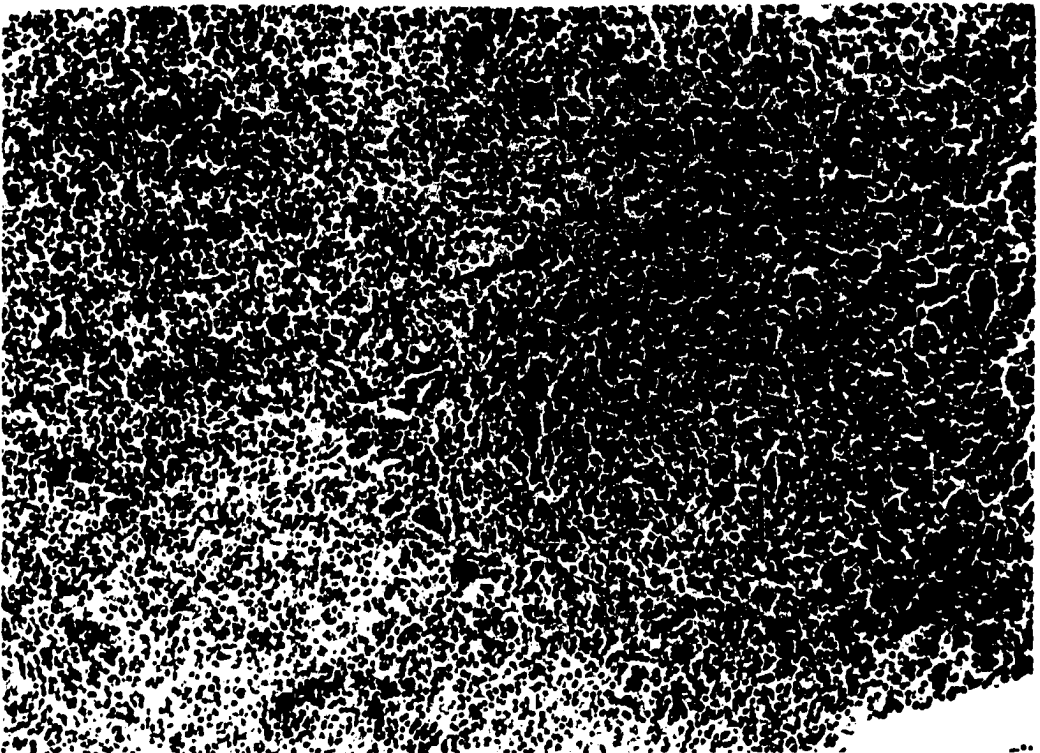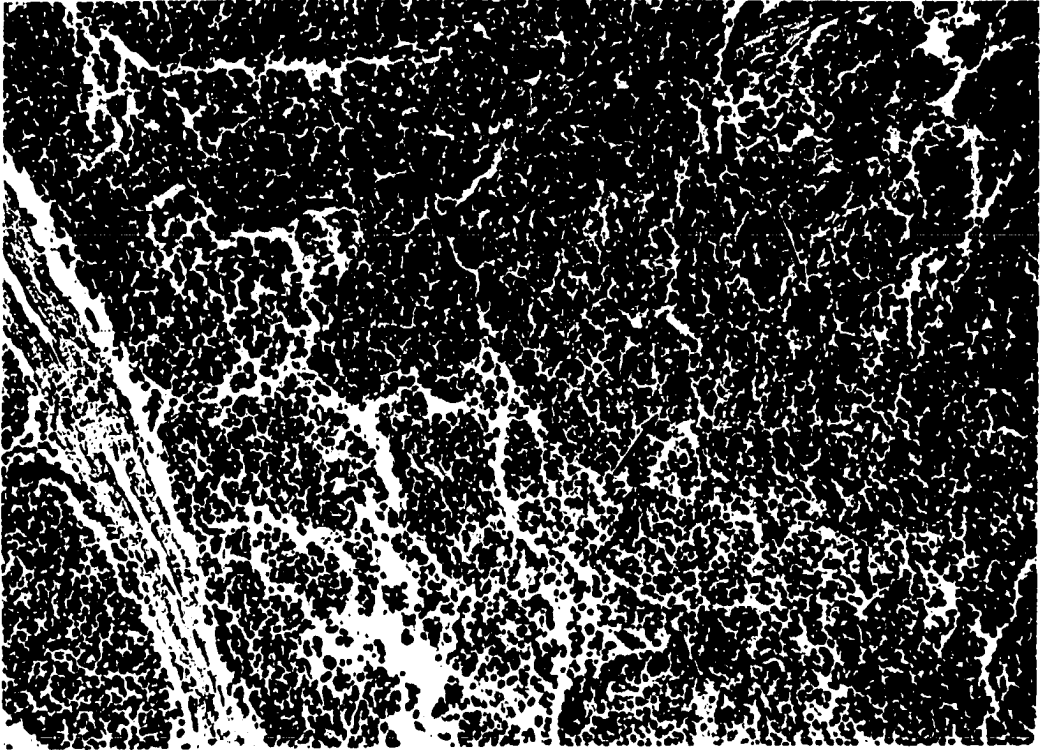   exercise.   x 156.   H. and E. stain.

Figure 36. Bronchial lymph node from a dog exposed to
yttrium oxide for 30 days. Arrows point to some
of the many dust-laden macrophages. x 156.
H. and E. stain.

Figure 37. Bronchial lymph node from a dog exposed to
yttrium oxide for 30 days. Arrows point to a
cluster of macrophages located at the periphery
of a germinal center. x 156. H. and E. stain.

mesenteric lymph nodes, testis or ovary, kidney, adrenal, and bone marrow showed that these organs remained normal.

Radiographic Examination

In a report of the United States Atomic Energy Commission (1964) it is noted that radiographic lesions, observed as a thickening of the air passages around the hilus of the lungs, are produced in dogs exposed to yttrium oxide. The thickening is progressive and extends peripherally with increasing exposure. In the above work the first radiographic examination was accomplished after approximately 50 exposure days (300 exposure hours). Slight radiographic lesions were apparent from these first radiographs.

In this experiment, where the total exposure time was 180 hours, an attempt was made to determine if radiographic lesions were apparent as a result of this shorter exposure period. The postexposure radiographs were compared with the radiographs obtained prior to exposure. Very subtle changes were apparent and consisted of slightly increased density at the hilus. It is possible that the enlarged bronchial lymph nodes which contained numerous dust-laden macrophages contributed to the slight increase in density noted.

SUMMARY

The purpose of this experiment was to determine the effects of exercise and inhaled yttrium oxide on blood lactic acid, erythrocyte volume, plasma volume, hemoglobin, total leukocyte count and lung histology in dogs. It was supposed that interference with oxygen transfer, transport or utilization, induced by yttrium oxide would be reflected in increased levels of postexercise blood lactate by virtue of an increase in anaerobic glycolysis.

Twenty-four beagle dogs were trained to run on a treadmill. Following their training, 18 dogs were exposed to a chamber atmosphere containing yttrium oxide for six hours/day, five days/week for six weeks. The yttrium oxide aerosol in the chamber atmosphere was a dust in which 74 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. The remaining six dogs were placed in chambers for the same time period but to which no yttrium oxide was added. These were to serve as histologic controls. During the exposure period, daily exercise was accomplished on a treadmill. A level of running was established prior to the exposure by measuring the blood lactic acid concentration within 30 seconds following a 10 minute treadmill exercise period. When an apparent plateau of lactate concentration was reached, no further

adjustments in speed were made. Prior to exposure erythrocyte volumes using Cr^{51}, plasma volumes using T-1824, hemoglobin concentrations, and leukocyte counts were determined on all exposed dogs. Throughout the exposure period, postexercise blood lactate concentrations were determined twice weekly. Following the termination of the 30-day exposure period the erythrocyte and plasma volumes, hemoglobin concentrations, and leukocyte counts were again determined.

Analysis of the data was accomplished by considering the animals in a paired experiment. Accordingly, the response was presented as the difference between the observation at the termination of the exposure to yttrium oxide and the observation at the beginning of the six-week exposure period.

The postexercise blood lactic acid concentration and leukocyte count were significantly increased. A slight significance was noted for the erythrocyte decrease and no significant difference was detected in plasma volume and hemoglobin concentration.

With regard to the postexercise blood lactic acid concentration increase, it appears that four to five dogs contributed primarily to its significance. In these dogs, a decrease in erythrocyte volume occurred. It is supposed that further increases may have been detected in other dogs if the exposure period had been lengthened. It was also considered that selection of runners with a better aptitude for treadmill running might be considered for future experiments. It would

then be possible to establish a greater work load prior to exposure and thus increases in postexercise blood lactate might be more readily attained. Under these conditions oxygen transfer, transport, and utilization would already be at the upper limit of compensation. There was no apparent reason for the erythrocyte volume decrease in dogs with elevated postexercise lactic acid. It is suggested that transfer of some yttrium oxide had taken place to other organs where it inhibited erythropoiesis. Such a condition may have been detected only where the tissue need for oxygen was accentuated with treadmill exercise.

Histologic changes noted in the lungs of dogs exposed to yttrium oxide were alveolar epithelial cell hypertrophy, hyperplasia, and desquamation. The desquamated alveolar epithelial cells were considered to be the free macrophages noted within the alveoli. Dust-laden macrophages were also noted within the enlarged bronchial lymph nodes of the exposed dogs.

It appears that concentration of blood lactic acid following treadmill exercise might be useful for the evaluation of materials where the toxicity becomes apparent when a greater functional activity is demanded of certain systems of the body.

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