The Effects of Aerosolized Lead on the Immune and Hematopoietic Systems

A. Nuri Ozkan

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THE EFFECTS OF AEROSOLIZED LEAD
ON THE IMMUNE AND HEMATOPOIETIC SYSTEMS

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science
Major in Microbiology
South Dakota State University
1981
THE EFFECTS OF AEROSOLIZED LEAD
ON THE IMMUNE AND HEMATOPOIETIC SYSTEMS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I wish to express my appreciation to Dr. Robyn Hillam for the extremely helpful advice and guidance during the course of this academic endeavor and in the preparation of this thesis.

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ABSTRACT OF THESIS

"The Effects of Aerosolized Lead on the Hematopoietic and Immune Systems"

The immunological responsiveness of two groups of mice exposed to 2.5 mg/m³ aerosolized Pb(NO₃)₂ either since birth or for 14 days prior to immunization were compared with mice receiving equivalent quantities Pb(NO₃)₂ (125 μg/mouse/day) by gastric intubation for 14 days. Each group was immunized at 5 weeks of age with 10⁸ sheep red blood cells to examine both primary and secondary antibody responses. Lead immunotoxicity was evaluated by comparing total and differential leukocyte counts, cellulose acetate electrophoretic serum profiles, phagocytic index, hemolytic complement activity, hemagglutination antibody titers and organ (lung, thymus, spleen, kidney and liver) to body weight ratios. Atomic absorption spectrophotometry was used to determine the concentration of lead in serum, blood and tissues. None of the immunological parameters examined were significantly affected by lead ingestion. However, aerosolized lead resulted in significant decreases in antibody titers; hemolytic complement activity; phagocytic indices; thymus to body and spleen to body weight ratios; and reduced numbers of monocytes as well as a neutrophilic shift to lymphocytes. Effects were greatest in mice born and reared in the aerosolization chamber. These results indicate the greater immunotoxicity of aerosolized lead compared with ingested lead and thus the daily health hazard aerosolized lead presents.
PROPOSED RESEARCH METHODS

The goal of this research was to compare the chronic immunological effects of aerosolized lead with that of injected lead.

An outbred strain of Swiss Webster mice originally obtained from Charles River Laboratories was used throughout this project. Each litter was sexed, distributed equally among groups, and cross-fostered. The effects of chronic aerosolized lead exposure were determined using mice exposed either for 14 days or since birth to aerosolized lead in an aerosolization chamber. Mice which received orally administered lead received an equivalent amount of lead by gastric intubation for 14 days. Both groups of mice had separate control groups. Since the immune and hematopoietic systems are extremely sensitive to chemical toxicity, immunological alteration was deemed to be an effective means of measuring the potential pollutant health hazards caused by lead.

The parameters used to determine immunological effects included:
1) total and differential leucocyte counts; 2) hematocrit values;
3) serum profile determinations using cellulose acetate electrophoresis; 4) antibody levels produced against sheep red blood cells;
5) measurement of hemolytic complement activity; 6) LD50 determinations after intraperitoneal challenge with Salmonella typhimurium;
7) phagocytic indices of both alveolar and peritoneal macrophage;
8) liver, lung, thymus, spleen and kidney to body weight ratios; and
9) atomic absorption spectrophotometric determinations of the lead concentrations in the above mentioned organs.
OBJECTIVES

The objective of this study was to assess the potential immunotoxic effects of aerosolized lead. This research compared the chronic immunological effects of aerosolized lead with that of ingested lead and determined that aerosolized lead at a subclinical level has a more deleterious effect on both the immune system and the hematopoietic system than does ingested lead.
STATEMENT OF SIGNIFICANCE

Lead is a stable chemical element yet it is one of the most insidiously toxic metals known to man. The cumulative biological effects of environmental lead pollution present a significant hazard to all aspects of life. This danger should be a concern for the health scientist and the environmentalist, as well as for the agriculturist and urban dweller.

There are two natural pathways for lead to enter the body, inhalation and ingestion. Aerosolized lead is mobilized into the atmosphere as a result of the high temperature combustion of fossil fuels and exists largely as inorganic lead particles of variable size. Some of these particles are small enough to allow for deep penetration into the pulmonary system while the larger particles are cleared by pulmonary cilia and ultimately swallowed. Lead ingestion can occur during the consumption of tinned foods or grains and dairy products contaminated with urban dusts.

The major emphasis of previous lead toxicity studies dealt with the effects of maternal lead ingestion on fetal development and the effects of ingested lead on children. These investigations dealt with extreme lead toxicity resulting in miscarriages and neurological problems caused by high doses of lead. Only recently have the more subtle changes caused by low levels of lead concentration been investigated.

Since the immune system is very sensitive to environmental pollutants, it provides an excellent means by which to study the effects of subclinical lead intoxication.
INTRODUCTION

The toxicity caused by ingestion of lead has been a concern for man throughout history. Only recently with the introduction of the industrial revolution has the need arisen to monitor the effects of subclinical lead exposure on human health.

Recent investigations on the subtle effects of subclinical lead toxicity have revealed ultra-structural changes such as the formation of inclusion bodies and metabolic changes such as reduced respiratory ability and inhibition of enzymatic activity. The ability of lead to mimic the metabolism of calcium can interfere with skeletal development, inhibit calcium dependent enzymes and replace calcium at calcium dependent membrane receptor sites.

Previous studies have demonstrated that ingestion of subclinical amounts of lead can lead to suppression of antibody synthesis, increased susceptibility to endotoxin and alterations in the phagocytic ability of macrophages.

Since previous research has concerned itself primarily with the study of ingestion of substantial quantities of lead, the following research has been conducted in an effort to understand the consequences of lead inhalation on health. Immunological impairment was an effective means of measuring the potential health hazards caused by lead due to the extreme sensitivity of the immune and hematopoietic systems to chemical toxicity.
1. **Sources and Emissions of Lead**

The extreme toxic effects of lead have been known for many years. Until recently, researchers have been concerned primarily with the clinical manifestations of severe lead toxicity on both the nervous and hematopoietic systems. With increased industrialization and motorized vehicle activity, and the subsequent increase of lead in the environment, emphasis is now being placed on the more subtle effects of lead. One active area of study is the immunotoxicity produced by lead (8).

Two major pathways exist for the entry of lead into the body: ingestion and inhalation. The primary route is ingestion; for example, the consumption of tinned foods containing high concentrations of lead (8). The Environmental Protection Agency (18) has estimated that the daily dietary intake of lead by human males ranges from 100 to 500 \( \mu g \) Pb/day. In the United States the incidence of lead poisoning by ingestion is alarmingly high; in 1969, 727 documented cases of lead poisoning were reported in New York City alone (8, 24). The majority of the ingested lead is in the elemental form and is rapidly excreted. Children afflicted with pica, resulting from the ingestion of paints containing lead chromate, account for a large percentage of the reported lead poisonings. Unlike the majority of ingested elemental lead which is rapidly eliminated, lead chromate is readily absorbed and stored by the body.

Particulate lead, contained in the exhaust gases of combustion engines, is the other major source of lead pollution. Levels of particulate lead are monitored by the Environmental Protection Agency
(18). The acceptable level of atmospheric lead, as determined by the EPA, is not to exceed 2 \( \mu g \) Pb/m\(^3\). However, lead levels much higher than the acceptable standard have been recorded. During peak traffic hours in Los Angeles, Fishbein et al. (20) reported lead air concentrations as high as 71.3 \( \mu g \) Pb/m\(^3\). This particulate lead exists largely as inorganic lead particles which vary in size. Much of the particulate lead is small enough (0.18 - 0.9 \( \mu m \)) so as to allow for deep and rapid penetration into the pulmonary system (8, 18, 24). Larger particles are cleared by the mucociliary escalator and ultimately swallowed.

2. Biological Effects of Lead Exposure

2.1.1 Metabolism of Ingested Lead

The metabolism of inorganic lead depends upon a number of intrinsic factors. These factors include: dietary calcium, sex, and the types of tissue involved.

Low levels of dietary calcium have resulted in increased lead absorption due to the competitive nature between lead and calcium (4). Lead replaces calcium at enzyme receptor sites via a chelating type effect (donor-acceptor). This binding appears to be principally a covalent type bonding rather than ionic (18).

Barry et al. (5) have shown that sex influences the metabolism of lead. Non-occupationally-exposed adult males have 30% higher lead levels (164.8 mg) in both bone and soft tissue than do adult females (103.6 mg). With occupational lead exposure the total mean lead body burden increased to 566.4 mg in adult males.
Lead levels vary considerably depending upon the type of tissue; for example, lead levels in the aorta are generally 20 times higher than those in the muscle. Using a modified graphite tube furnace technique, Schlick et al (49) determined lead concentrations of various tissues. They found that approximately 100 mg of orally administered lead acetate/kg body weight/day was the threshold dose required for a long-term increase in tissue Pb concentration. However, after 30 days treatment, neither the threshold dose nor 10 times this dose was sufficient to elevate the blood lead concentration, indicating rapid clearance of lead from the blood. Within three days after intravenous (IV) injections of a subclinical dose of lead, blood lead levels returned to control levels.

More than 90% of the total lead body burden in adults is immobilized in bone; the remaining lead is localized in soft tissue. The Schlick study revealed that the concentration of lead in the soft tissues (liver and kidney) returned more slowly to normal than did blood lead levels but that lead bone content remained elevated for an extended period of time. Schlick et al interpreted the data to indicate that the soft tissue function as an intermediate storage area, whereas the bone acts as a long term depot. The subclinical concentration of lead used in the Schlick study has been shown capable of producing functional disorders of the reticulo-endothelial system, inhibition of the enzyme responsible for heme synthesis (ALA-d) and neurophysiological alterations. Since these disorders occur without producing elevated blood lead levels, the determination of blood lead level alone, may be inadequate for reliable measurement of lead accumulation (13, 14, 49, 50, 57).
Similar experiments using monkeys yielded comparable results (57). The mechanism and pathway of lead mobilization during pregnancy were determined by Keller and Doherty (30). Pregnant mice were administered Pb in their drinking water. Results indicate that lead was primarily sequestered in maternal bone but that during gestation small amounts were also transferred directly to the fetus. During lactation lead was mobilized and subsequently transferred to suckling pups. Using these results as a model of lead mobilization, they concluded that approximately 5 mg lead would be transferred during the period of human lactation.

2.1.2 Metabolism of Aerosolized Lead

Whereas approximately 10% of ingested lead is absorbed, nearly 48% of inhaled lead is absorbed (12). The ultimate fate of inhaled particulate lead is dependent upon a number of physical and chemical factors which include: solubility, diffusibility, protein binding capabilities, chemotactic ability and size of the particle (11, 29, 1, 45).

Chamberlain et al (12) reported that after a 50 hour post aerosolization period of lead (0.5 μm), 48% of the initial lung burden had been transferred to the blood. Excretion in fecal material after inhalation was very slight, indicating that only a small amount of lead was cleared by the pulmonary cilia and swallowed. Renal clearance was extremely high and histological changes in tissue could readily be seen.

Kehoe (29) reported that at low lead air concentrations a large portion of the particles trapped in the upper respiratory tract
with lead toxicity. Anemia is associated with both severe lead intoxication as well as mild environmental lead exposure (41, 55). Lead-induced anemia is due to the inhibition of the enzyme delta aminolevulenic acid dehydratase (ALA-d), a required enzyme for heme synthesis (2). This impairment may be due to the binding of lead directly to the enzyme rendering it functionally inactive.

Another example of lead-induced enzyme dysfunction is the binding of lead to nucleosides. This binding results in improper coding for protein synthesis. An example of this type of dysfunction is in the synthesis of certain enzymes required for oxidative metabolism (9, 22, 23).

2.3 Immunotoxic Effects

2.3.1 Cellular Effects

The effects of lead exposure on cellular viability and function have only recently been determined. These effects range from decreased enzymatic activity to lowered cellular replication.

Kussel et al (40) demonstrated the effects of lead nitrate on normal proliferation of cultured cells. A definite inhibitory effect on the in vitro growth of a rat liver cell line, neuroblastoma cells and two glioma cell lines were reported with the addition of 10^{-5}M lead nitrate to the culture medium.

Mitogen-induced lymphocyte proliferation is inhibited with in vivo lead exposure. In vivo exposure to subclinical doses of lead inhibited the proliferative response of lymphocytes treated with lipopolysaccharide (a B-cell mitogen), purified protein derivative (a T-cell mitogen) or phytohemagglutinin (a T-cell mitogen) (17, 39, 51).
Similarly, Koller and Roan (37) observed that the mixed lymphocyte culture reaction (MLR) using splenic lymphocytes from mice exposed to lead orally was not significantly altered.

2.3.2 Effects on B-Lymphocyte Activity

Oral exposure to subclinical doses of lead or cadmium resulted in significant impairment of B-cell function. The B-cell is directly responsible for antibody formation. Koller and Kovacic (35), using a modification of the Jerne plaque assay, reported that Swiss Webster mice exposed to subclinical doses of orally administered lead acetate, demonstrated a marked decrease in antibody forming cells. Inhibition was evident in both the primary and secondary immunologic response, the primary response being the first immunologic response, composed primarily of IgM antibody; the secondary response is the memory or anamnestic response where largely IgG is the predominant antibody. The greatest impairment was observed on the secondary (IgG) response.

In a similar study Koller, Exon and Roan (33) reported that lead administered by gastric intubation or injected intraperitoneally (IP) in mice stimulated the formation of 19S (IgM) antibody but caused a notable reduction in 7S (IgG) antibody in splenic lymphocytes.

Faith and Kimmel (19) reported a decrease in B-cell function after chronic pre- and postnatal oral exposure of low levels of lead acetate in female CD rats. The total antibody levels of both the primary and secondary antibody response were markedly depressed in both pre- and postnatal exposure groups.
2.3.3 Effects on Macrophage

Macrophage play a key role in the defense mechanism of cellular immunity by inactivating and processing foreign particles and bacteria. The alveolar macrophage is the single most important factor involved in the removal of inhaled soluble particles (11, 28, 45, 56).

Exposure to lead has resulted in a number of adverse effects on macrophage. Effects of lead exposure on macrophage include such findings as reported by Bouley et al. (7) who found reduced phagocytic activity in alveolar macrophage as measured by bacterial clearance and inactivation of aerosolized bacteria, in mice receiving aerosolized lead. Kiminski and co-worker (28) have shown that a high order of toxicity occurs when pulmonary macrophages are directly exposed (in vitro) to lead. Kiminski (28) also found that the reduced functional activity of macrophages following lead exposure results in the inhibition of oxidative metabolism, which is a prerequisite for intracellular bacteriocidal activity. In addition, Trejo (54) demonstrated that a single intravenous injection of lead acetate impaired the phagocytic ability of Kupffer cells. Electron microscopic examination of alveolar macrophage from mice exposed to aerosolized lead revealed structural damage to mitochondrial and endoplasmic reticulum which would alter enzyme synthesis and phagocytic ability (6).

2.3.4 Effects on Acquired Immunity

Numerous investigators have reported that exposure to lead can result in reduced resistance to certain bacterial and viral infections. Specific effects such as decreased antibody
production, impaired phagocytic ability and impaired detoxification by
the liver have been reported (6, 7, 26, 35).

Hemphill et al (25) found that mice injected IP with subclinical
doses of lead nitrate over a 30 day period showed greater suscepti-
bility to IP challenge with *Salmonella typhimurium* than controls.
Selye (52) found that rats which are ordinarily resistant to certain
bacterial endotoxins were more susceptible to the action of these
toxins when exposed to 1 mg lead acetate/100 g body weight admini-
stered IP. Similar results were obtained by Cook, Hoffman and DiLuzio
(16). A single IV injection of 2 mg lead acetate/100 g body weight
resulted in an enhanced susceptibility of Charles River rats exposed
to *Escherichia coli* by IP challenge.

Lowered pulmonary resistance to bacterial infection has been shown
with lead exposure (7). Mice were administered lead via aerosoliza-
tion until a lung lead burden of 6 mg/mouse was achieved. Mice were
then challenged with *Pasteurella multocida* via aerosolization. Lead-
exposed mice had an impaired immunological response which resulted in
decreased resistance to the challenge bacteria.

Lowered resistance to viral infection in lead exposed animals has
also been documented. Koller (33) demonstrated an immunosuppressive
effect in rabbits exposed to 2500 ppm lead acetate given in drinking
water for 70 days. Rabbits were then immunized with pseudorabies
virus. The antibody titers to the virus, as measured by the serum
neutralization plaque assay, were significantly lower in the lead
exposed animals than in the control animals.
2.3.5 Effects on Complement Activity

Complement is one of the most essential non-specific humoral factors in immunity. Complement is involved in the lysis, opsonization and inactivation of cellular, bacterial and viral antigens. Should the complement system be impaired, bacterial and viral virulence would be enhanced.

There are two pathways by which the lytic ability of complement may be fully activated. These pathways, the classical pathway and the alternate pathway, differ in the method of initial activation. The classical pathway depends upon the operation of nine protein components (C1-C9) after activation by an antigen-antibody reaction. The alternate pathway is activated by a C3 convertase, an enzyme which is triggered by extrinsic agents such as endotoxin or microbial polysaccharides. C3 convertase activates the alternate complement cascade starting with complement component C3 rather than C1 as is the case in the classical pathway.

Decreases in complement activity after exposure to various heavy metals have been widely reported. Montgomery et al (44) reported that zinc, copper and manganese at concentrations greater than 100 μM strongly inhibited complement-mediated hemolysis of antibody-sensitized sheep red blood cells (srbc). This inhibition appeared to be due to the decreased formation of activated erythrocyte-antibody-complement complexes. This theory was subsequently confirmed by Yamamoto and Takahashi (58) who found that both zinc and copper ions prevented formation of an active terminal complex by inhibiting C9 binding.
In a similar study Montgomery, Chvapil and Zukoski (43) reported the in vitro effects of a wide range of zinc chloride concentrations (25 -500 μM) on the activity of both free and cell-bound components of guinea pig complement. It was shown that all components were found to be inhibited by super-physiological concentrations of zinc (>100 μM). In order for inhibition to occur, the addition of zinc had to be as a third reactant directly rather than addition after complement was cell bound. In addition, zinc did not cause irreversible denaturation of any component of complement. Studies by Muller-Eberhard et al (46) have shown that zinc and cadmium can block C2 activation, thereby terminating the complement cascade at an early stage in the classical pathway and disrupting lysis.
MATERIALS AND METHODS

1. Animals and Treatment Design

1.1 Animals

An outbred strain of Swiss Webster mice originally obtained from Charles River laboratories was used in this study. Animals were obtained from a breeding colony at South Dakota State University, Department of Microbiology. Food (Mouse Chow #5015 Ralston Purina) and water were supplied ad libitum throughout all the experiments.

Unless stated otherwise, Pb exposures began when the mice were 14 days of age. Litters were sexed, randomly distributed into groups of 10 mice, and cross-fostered.

1.2 Experimental Design

1.2.1 Oral Lead Administration

The lead ingestion group was administered lead orally via gastric intubation using a 1/4 cc graduated glass tuberculin syringe (Becton-Dickinson) and a balled bio-medical animal feeding needle (Perfekturn, Inc.). The lead ingestion group received a daily dosage of 125 µg lead nitrate (Pb(NO₃)₂) in 0.1 cc deionized water. Control mice received an equivalent quantity of deionized water. Animals were treated for 14 days.

1.2.2 Lead Aerosolization

The lead aerosolization study contained two groups. The first group was placed into the aerosolization chamber at 14 days.
of age. The second group was born and reared in the chamber. The chamber design was similar to that described by Arvik et al. (3). The exposure chamber consisted of an ILE glovebox with an exhaust filter fan. Filtered air (air was from our "air valve") and a solution of 1000 ppm Pb(NO₃)₂ were forced through a nebulizer producing a liquid aerosol which was blown into a heated desolvation chamber. The spray entering the chamber was flash evaporated to produce water vapor and particulates composed primarily of lead. This passed into a condenser cooled to approximately 8°C. The larger dry particles of lead were removed by collision with the walls of the condenser spiral. The smaller sized particles passed through the spiral and entered the exposure chamber via a short piece of tygon tubing. Controls for this portion of the study remained outside the chamber.

Mice were exposed to aerosolized lead for 14 days in the case of the two week old mice and four weeks in the case of the mice born and reared in chamber.

Both groups were exposed to 2.5 mg Pb(NO₃)₂/m³ or approximately 80 μg Pb/mouse/day. This value was determined using data on the lung volume capacity and respiration rate of mice (47).

It was determined with the use of an Andersen Cascade Impactor that 81% of the lead entering the chamber was of size <2 microns, allowing for deep penetration into the pulmonary system.
2. Methods

2.1 Immunological Methods

2.1.1 Immunization

Sheep red blood cells from the same sheep and obtained no later than 7 days prior to immunization were used for all injections. Whole sheep blood was collected by jugular venipuncture in sterile 2 liter Erlenmeyer flasks containing 40 ml of 40\% sodium citrate. After collection the srbc were transferred to sterile screw cap bottles and stored at 4°C. For injection the erythrocytes were washed three times in 0.85\% saline and diluted to the desired concentration.

After fourteen days of treatment, and at four weeks after birth in the case of the chamber born mice, each group of mice was injected intraperitoneally (IP) with $10^8$ srbc in 0.25 cc sterile saline. On day 18, half of each group was anaesthetized with ether and terminated by exsanguination and cervical dislocation. The remaining mice were reinjected as previously described on days 18 and 22 and terminated on day 25 to determine the anamnestic or secondary immune response.

2.2 Procurement and Treatment of Tissues, Blood and Serum

Mice were anaesthetized with ether prior to exsanguination. Blood was collected by severing the subclavian artery. A sample of blood was allowed to clot for approximately 1 hour at room temperature. Using a wooden applicator stick, the clot was removed from the walls of the glass tube and dried for lead determinations. The serum was clarified by centrifugation and frozen until needed. Blood samples for total leukocyte counts, differential white blood
cell determinations and hematocrits were also taken. After termination the mice were weighed and the thymus, lung, liver, kidney and spleen were removed, weighed and stored frozen in plastic vials for lead analysis by atomic absorption spectrophotometry.

2.2.1 Hematocrit

Heparinized microhematocrit capillary tubes were filled with whole mouse blood and centrifuged in an Adams Hematocrit centrifuge to determine the percentage volume of erythrocytes.

2.2.2 Total Leukocyte Counts

Forty microliters of whole mouse blood from each mouse were added to separate disposable blood dilution vials containing 20 ml of Isoton II diluting buffer (Coulter Diagnostics, Inc.). Five drops of Zap Isoton II were added to lyse erythrocytes. Leukocyte counts were made on a Model F Coulter Counter (Coulter Electronics). Two separate counts were taken, averaged and corrected.

2.2.3 Differential Blood Counts

Blood smears were made using a 3" x 1" bev-1-edge micro slide and stained in Camco Quick Stain using the recommended procedure. A total of one hundred leukocytes was counted and differentiated into monocytes, lymphocytes and neutrophils.

2.2.4 Cellulose Acetate Electrophoresis

Cellulose acetate electrophoresis was performed to determine the relative proportion of the major serum components. Titan III cellulose acetate strips (Helena, Inc.) were submerged in
Electro HR electrophoresis buffer, pH 8.6 - 9.0 (Helena, Inc.), until saturated. An aliquot of 2 µl serum was applied to one end of a strip using a sample applicator. The slides were electrophoresed at 180V for 30 minutes at room temperature. The slides were stained in Ponceau S stain for 5 minutes, and destained in 3 consecutive baths of 5% acetic acid. Clearing of the strips was accomplished by submerging them in a Sepra Clear II cellulose acetate clearing solution and heating in an 80°C oven until clear. The relative proportions of the major serum components were measured using a planimeter to determine the area of each component peak obtained from densitometer scans of the strips.

2.2.5 Direct Hemagglutination

All sera were tested for the presence of anti-srbc IgG and IgM antibodies using direct hemagglutination. These tests were performed using a modified microtiter assay similar to that of Stavitsky (53). The assay was performed in 96-well conical bottom microtiter plates (Linbro). All samples were run in duplicate in the following manner. Normal rabbit serum (1:100) in phosphate buffered saline (PBS), pH 7.4, was added to each well of the microtiter plate. The serum to be tested was added to the first well of each row and two-fold dilutions were made through the eleventh well using a micro-dilutor (the twelfth well contained only PBS). To each of the wells an equal volume of 2% srbc suspension was added. After a two to four hour room temperature incubation, the results were recorded.

Agglutination due to both IgG and IgM was obtained using 1:100 NRS in PBS as the diluent. Agglutination due to IgG antibody was
determined using a 1:100 NRS-PBS diluent containing 0.2 M beta-mercaptoethanol. Beta-mercaptoethanol is a reducing agent which selectively cleaves the disulfide bridges in the IgM molecule, leaving the IgG molecule functional. Titers were reported as the reciprocal of the last dilution showing any agglutination. A negative result was indicated by a "button" of nonagglutinated cells.

2.2.6 Complement Hemolytic Activity Determination

To determine the effect of in vivo and in vitro lead exposure on the hemolytic activity of complement, the following assay was performed.

2.2.6.1 In vitro lead exposure

Reconstituted whole guinea pig complement (C') (BBL) was used throughout this procedure. The optimal concentration of complement, as determined by the 50% endpoint method of Burrell (10), was 5 CH50 units. Two units of whole hemolysin (2H') (BBL) were used as the optimal hemolysin concentration for the sensitization of sheep erythrocytes.

Lead solutions used in the assay were lead acetate, lead chloride and lead nitrate diluted in isotonic saline at concentrations ranging from 25 μM to 400 μM. All samples were assayed in triplicate.

Hemolytic assays were conducted by mixing in a clean 12 x 75 mm glass tube 1.5 ml of the test solution, 0.5 ml of the hemolysin solution containing 2 whole H' units, 0.5 ml 2% srbc, and 0.5 ml complement containing 5 CH50 units. The mixture was incubated at 37°C for 30 minutes with frequent agitation. After incubation the tubes were
centrifuged for 5 minutes and the optical density of each supernatant was measured at 541 nm to determine the amount of oxyhemoglobin released. The percent hemolysis was calculated by using the following formula:

\[
\text{Percent Hemolysis} = \left( \frac{\text{Absorbance 541 nm of test sample}}{\text{Absorbance 541 nm of 100\% hemolysis control}} \right) \times 100
\]

2.2.6.2 In vivo lead exposure

Blood from 8 mice receiving aerosolized lead for 2 weeks and 8 control mice was collected in pre-chilled test tubes and placed immediately in an ice bath until clotted. Serum was obtained as previously described and assayed the same day to avoid loss of C' activity. A mixture consisting of 150 µl of a 1:2 dilution of the serum in pH 7.3 veronal buffered saline (VBS), 0.5 ml hemolysin containing 3 H' units diluted in VBS, 0.5 ml 1% srbc, and 1.85 ml of VBS were added to clean 12 x 75 mm glass test tubes and vortexed. Samples were then incubated at 37°C for 30 minutes with frequent shaking. After incubation the tubes were centrifuged and the optical density of the supernatant was measured at 541 nm. To correct for any hemolysis present in the mouse serum itself, 150 µl of a 1:2 dilution of serum was brought up to 3 ml in VBS and the optical density measured and subtracted from the final absorbance of the test sample.

3. Biological Assays

3.1 Phagocytic Index

The phagocytic index was performed to evaluate the \textit{in vitro} phagocytic ability of both the alveolar and peritoneal
Alveolar macrophages were harvested in the following manner. In order to prevent trauma to the lung, mice were euthanized by injecting 0.3 ml of a 1:10 dilution of sodium pentobarbital (64 mg/ml). The trachea was exposed and a 16-gauge 2.5 inch Catholon IV Catheter Placement Unit (Jelco Labs, Inc.) was inserted between the tracheal rings and secured. The lungs were lavaged using three 1 ml washes of sterile saline. The thoracic region was massaged externally for approximately 3 minutes prior to saline withdrawal and lavage fluids pooled. An average of 2.5 ml of lavage fluid was routinely obtained. Alveolar macrophages were sedimented by centrifugation at 400 g for 15 minutes at room temperature and resuspended in 1 ml of medium 199, Hanks base (BBL) supplemented with 20% heat-inactivated rabbit serum. Viability and cell numbers were determined by the trypan blue exclusion method (Appendix A).

Peritoneal macrophages were harvested by washing the abdominal area with ethyl alcohol and carefully exposing the peritoneal sac. Three milliliters sterile saline were injected into the peritoneal cavity, massaged for 2 minutes and removed. This procedure was repeated and the peritoneal washes pooled. The macrophages were then sedimented, resuspended and counted as previously described.

The technique used for the phagocytic index was a modification of the procedure used by Gardner et al (21). Fluorescent yellow 0.8 μm diameter polystyrene latex beads (Dow Chemical) were added to obtain an 18:1 latex sphere to macrophage ratio. The samples were incubated in a 37°C water bath for 30 minutes with frequent agitation. Following incubation, two 20 μl samples of the cell suspension were placed into
each of the 2 etched rings of a 3" x 1" fluorescent antibody (FA) slide, air dried, and fixed in Bouins fixative (Appendix B) for 10 minutes. Following fixation the slides were washed in 50% ethyl alcohol for 3 minutes, placed into a 100% xylene bath for 3 hours with occasional shaking to dissolve the extracellular latex spheres, and rinsed with 50% ethanol. Macrophages were stained by placing the slides in a 0.001% fluorescent acridine orange saline staining solution, pH 10, for 4 to 5 minutes and rinsed. A drop of Bacto FA Mounting Fluid (Difco, Inc.) was added to the two samples on each slide and covered with a Fisher brand 2 x 2 mm #1 coverslip. The number of ingested beads was counted using a Leitz Dialux UV scope with mercury cathode using the 40X and oil immersion objectives. The phagocytic index was calculated using the method of Carpenter (10). The number of latex beads phagocytized by ten macrophage from each mouse was used to determine the phagocytic index of each exposure group and respective control.

3.2 LD50 Determination

The effect of lead exposure on the susceptibility to bacterial challenge was determined by the intraperitoneal injection of serial dilutions of a 12-18 hour culture of *Salmonella typhimurium* into mice receiving subclinical amounts of lead. Mice were weighed and evenly distributed among groups according to weights in a random fashion. Six mice were injected with each dilution. The number of dead mice per dilution was recorded daily for 1 week. The LD50, standard error and 95% confidence limits were calculated by the method of Irwin and Cheeseman (27).
4. Chemical Methods

4.1 Determination of Lead Content in Tissues

Lead content in blood, serum, spleen, liver, lung and thymus was analyzed by atomic absorption spectrophotometry using a modification of the procedure used by Christian et al. (15). Following initial drying at room temperature, the previously collected tissue samples were dry-ashed at temperatures not exceeding 500°C. The spleen, lung lobe, thymus and blood samples were prepared as whole samples. Due to the mass of the liver and kidney, it was necessary to grind and weigh a portion of the sample being analyzed. Each sample was added to a 30 ml Kjedahl flask rinsed with 3 N hydrochloric acid. To each flask was added 5 ml of double distilled nitric acid. The mixture was boiled until approximately 2 ml of the nitric acid remained, at which time 250 μl of perchloric acid and 2-3 ml of nitric acid were added and boiled to near dryness. The sample was solubilized by adding 1 ml of deionized water to the flask, heating and then transferring it to a 5 ml volumetric flask and bringing it up to volume.

Atomic absorption spectrophotometric analysis was performed using a Perkin Elmer model 503 AA spectrophotometer using argon as the carrier gas. The temperature and time settings were 30 seconds drying time at 150°C; 50 seconds charring time at 550°C; and 7 seconds atomizing time at 2300°C. The wavelength setting used was 283.7 nm, and the lead cathode was set at 10 mA. Parts per million lead was determined using a standard curve of 0.05, 0.1, and 0.15 ppm lead solutions. All test samples and controls were run in triplicate.
5. **Statistical Analysis**

All statistical analyses were performed with a Texas Instrument 58C calculator. Data were evaluated by the one way analysis of variance. Levels of significance are reported at the 99 and 95% levels.
RESULTS

1. Lead Exposure

1.1 Aerosolization

The level of lead nitrate used for aerosolization was 2.5 μg/m³ as determined using an Anderson Cascade Impactor. Using the lung volume and respiration rates for mice (47), it was determined that each mouse was inhaling approximately 80 μg Pb/day. At the lead concentration used, no overt clinical symptoms of lead toxicity were observed.

1.2 Oral Exposure

The level of oral lead exposure was chosen to correspond to the concentration of lead received by the lead aerosolized mice. The oral dosage of lead was 125 μg Pb(NO₃)₂/mouse/day. The two natural exposure routes of lead were compared to respective controls and to one another to assess the most immunotoxic route of exposure.

2. Effects of Lead on the Immune and Hematopoietic Systems

2.1 Hematocrit Values

Neither the oral lead exposed group (Pboral), the two week aerosolized lead group (Pbair), nor the chamber born group (Pbch) had significantly different hematocrit values when compared to control group Coral (deionized H₂O gavage) and Cair (non-aerosolized). Since clinical lead toxicity has been shown to result in major hematocrit changes, the dosage of lead administered in this experiment was of a subclinical level.
2.2 Organ to Body Weight Ratio

Organ to body weight ratios were performed to determine the effects of lead on specific lymphoid organs (Table 1).

Of the five organs compared (lung, kidney, liver, thymus and spleen) in the orally lead ingested group, no significant differences were seen.

In group Pbair highly significant decreases in organ to body weight ratios occurred in the thymus and spleen samples when compared to controls. In addition, a highly significant increase in the organ weight ratio of the kidney occurred in this group.

Organ to body weight ratios which occurred in group Pbch when compared to controls included highly significant decreases in spleen and liver samples, with a highly significant increase in the kidney to body weight ratios.

2.3 Total Leukocyte Counts

Total leukocyte counts were determined to assess the effects of subclinical in vivo lead exposure on white blood cells (WBC). The results are shown in Table 2. The total WBC count in group Pboral did not differ significantly from the oral control group receiving deionized water. The two week lead aerosolized group also had similar cell counts compared to the non-aerosolized control group Cair. A highly significant reduction was observed in group Pbch when compared to control group Cair. The longer lead exposure together with the cumulative nature of lead might account for the reduction in white blood cells.
<table>
<thead>
<tr>
<th>Group</th>
<th>(X) Kidney/Body</th>
<th>(X) Thymus/Body</th>
<th>(X) Spleen/Body</th>
<th>(X) Liver/Body</th>
<th>(X) Lung/Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control (Coral)</td>
<td>1.48 ± .07</td>
<td>0.40 ± .05</td>
<td>0.62 ± .08</td>
<td>6.28 ± .34</td>
<td>0.97 ± .05</td>
</tr>
<tr>
<td>Orally Fed Lead (Pboral)</td>
<td>1.46 ± .07</td>
<td>0.38 ± .08</td>
<td>0.62 ± .26</td>
<td>6.68 ± .24</td>
<td>0.94 ± .06</td>
</tr>
<tr>
<td>(75 µg Pb/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-aerosolized Control (Ca)</td>
<td>1.43 ± .07</td>
<td>0.39 ± .07</td>
<td>0.62 ± .25</td>
<td>7.48 ± .92</td>
<td>0.96 ± .11</td>
</tr>
<tr>
<td>Aerosolized Lead (Pbair)</td>
<td>1.63 ± .07b</td>
<td>0.27 ± .07b</td>
<td>0.46 ± .06b</td>
<td>6.91 ± .60</td>
<td>0.96 ± .10</td>
</tr>
<tr>
<td>(80 µg Pb/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamber Born (Pbch)</td>
<td>1.9 ± .19b</td>
<td>0.31 ± .04</td>
<td>0.41 ± .07b</td>
<td>5.76 ± .60b</td>
<td>1.05 ± .07</td>
</tr>
<tr>
<td>(80 µg Pb/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Mean ± S.D.
b \( p < 0.01 \) as determined by the Analysis of Variance method
c Ten mice were observed in each dosage group.
2.4 Differential Cell Counts

To determine changes in cell populations due to in vivo exposure to subclinical amounts of lead, differential cell counts were done. The results of the cell counts are listed in Table 2. No significant differences were observed between groups Pb\textsubscript{oral} and controls C\textsubscript{oral}. Comparing lead aerosolized groups Pb\textsubscript{air} and Pb\textsubscript{ch} to controls C\textsubscript{air}, a neutrophilic shift to lymphocytes was observed. A significant reduction in monocytes also occurred in group Pb\textsubscript{air}.

2.5 Cellulose Acetate Electrophoresis

Changes in serum globulins due to lead exposure were measured by cellulose acetate electrophoresis and are reported in Table 3. The serum profiles for group Pb\textsubscript{oral} were similar to Controls C\textsubscript{oral}, indicating no alterations in the serum proteins with this route of exposure. Serum profiles of the two week lead aerosolized group when compared to nonaerosolized controls, indicated a significant reduction in the gamma globulin peak. The gamma region is the electrophoretic region which contains antibody. The other electrophoretic regions (albumin, alpha and beta globulins) were within normal range. In group Pb\textsubscript{ch} a highly significant decrease in the gamma globulin region was noted as well as a decrease in the beta globulin peak, when compared to controls C\textsubscript{air}. The beta globulin region contains notable serum proteins such as plasminogen, fibrinogen, complement components, activators and inhibitors. The albumin region in group Pb\textsubscript{ch} increased to a highly significant degree to compensate for the decreases in gamma and beta globulins. The reduction in the gamma globulin region
Table 2

Differential and Total Leukocyte Counts
Of Mice Exposed to Lead by Ingestion and Inhalationc

<table>
<thead>
<tr>
<th>Group</th>
<th>(%) Lymphocytes</th>
<th>(%) Neutrophils</th>
<th>(%) Monocyte</th>
<th>Total Leukocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control (Coral)</td>
<td>73.3 ± 4.9</td>
<td>19.5 ± 2.5</td>
<td>7.2 ± 2.1</td>
<td>17,633 ± 5887/ml</td>
</tr>
<tr>
<td>Orally Fed Lead (Pboral) (75 μg Pb/day)</td>
<td>70.6 ± 8.4</td>
<td>23.2 ± 6.6</td>
<td>6.2 ± 4.4</td>
<td>16,275 ± 2310/ml</td>
</tr>
<tr>
<td>Non-aerosolized Control (Cair)</td>
<td>70.6 ± 5.4</td>
<td>23.2 ± 4.1</td>
<td>6.2 ± 2.2</td>
<td>19,840 ± 9354/ml</td>
</tr>
<tr>
<td>Aerosolized Lead (Pbair) (80 μg Pb/day)</td>
<td>79.8 ± 3.8a</td>
<td>15.8 ± 3.2a</td>
<td>4.4 ± 0.9a</td>
<td>19,950 ± 8227/ml</td>
</tr>
<tr>
<td>Chamber Born (Pbch) (80 μg Pb/day)</td>
<td>86.3 ± 3.4a</td>
<td>10.2 ± 3.2b</td>
<td>3.5 ± 0.8</td>
<td>16,475 ± 3768/mla</td>
</tr>
</tbody>
</table>

a p < 0.05 as determined by the Analysis of Variance method
b p < 0.01 as determined by the Analysis of Variance method
c Mean ± S.D.
d Ten mice were observed in each dosage group.
Table 3

Selected Effect of Lead on Serum Proteins
In Lead Exposed Mice\(^c\)

<table>
<thead>
<tr>
<th>Group</th>
<th>(%) Albumin</th>
<th>(%) Alpha</th>
<th>(%) Beta</th>
<th>(%) Gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control</td>
<td>62.6 ± 4.7</td>
<td>8.4 ± 2.0</td>
<td>7.6 ± 2.4</td>
<td>21.4 ± 8.0</td>
</tr>
<tr>
<td>Orally Fed Lead (75 µg Pb/day)</td>
<td>60.0 ± 6.4</td>
<td>11.3 ± 4.2</td>
<td>7.5 ± 4.0</td>
<td>21.2 ± 4.9</td>
</tr>
<tr>
<td>Non-aerosolized Control</td>
<td>56.1 ± 5.8</td>
<td>10.4 ± 2.9</td>
<td>7.6 ± 2.0</td>
<td>25.9 ± 4.1</td>
</tr>
<tr>
<td>Aerosolized Lead (80 µg Pb/day)</td>
<td>59.9 ± 4.8</td>
<td>11.9 ± 2.2</td>
<td>8.6 ± 3.3</td>
<td>19.6 ± 3.7(^a)</td>
</tr>
<tr>
<td>Chamber Born (80 µg Pb/day)</td>
<td>67.9 ± 2.5(^b)</td>
<td>10.5 ± 2.4</td>
<td>5.1 ± 1.3(^b)</td>
<td>16.5 ± 2.2(^b)</td>
</tr>
</tbody>
</table>

\(^a\) \(p < 0.05\) as determined by the Analysis of Variance method
\(^b\) \(p < 0.01\) as determined by the Analysis of Variance method
\(^c\) Mean ± S.D.
\(^d\) Ten mice were observed in each dosage group.
in both lead aerosolized groups is the first indicator in this study that the subclinical concentration of lead used is immunosuppressive when inhaled. Furthermore, it appears that with longer lead exposure, as with group Pb_{ch}, additional alterations in serum globulins can occur.

2.6 Direct Hemagglutination

The direct hemagglutination test (HA) combines high degrees of both sensitivity and specificity in measuring antibody to a specific antigen. The immunogen used in this study was srbc, which is both a T- and a B-cell immunogen. To differentiate the IgG antibody response from total antibody (IgG and IgM), β-mercaptoethanol was used. Hemagglutination titers for the orally ingested lead group (Pb_{oral}) were similar to control group C_{oral} receiving deionized water. This was the case for both the primary and secondary immunologic responses. These results compare favorably with the serum profile results for this group in respect to the 'normal' level of gamma globulin of group Pb_{oral}. With lead aerosolization, the HA titers for the primary immunologic response of both groups Pb_{air} and Pb_{ch} showed a highly significant decrease compared to controls C_{air}. The primary immunologic response is mainly due to 19S (IgM) antibody as determined with the use of β-mercaptoethanol. The HA titers for the secondary immunologic response in the two week lead aerosolized group Pb_{air} rose to an equivalent titer as the control group C_{air} for both total (IgG and IgM) and 7S (IgG) antibody. The secondary response is due primarily to IgG antibody. Secondary immunologic HA titers for group
Table 4
Hemagglutination Titers of Mice
After Pb Ingestion

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Antibody(^a) and 95% Confidence Intervals</th>
<th>Beta-mercaptoethanol resistant (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control: primary response</td>
<td>96 (73-115)</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Orally Fed Control: secondary response</td>
<td>2048 (1536-2816)</td>
<td>96 (81-105)</td>
</tr>
<tr>
<td>Orally Fed Lead: primary response (75 μg Pb/day)</td>
<td>96 (80-122)</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Orally Fed Lead: secondary response (75 μg Pb/day)</td>
<td>2048 (1536-2816)</td>
<td>128 (112-144)</td>
</tr>
</tbody>
</table>

\(a\) Titers expressed as the geometric mean.

\(b\) Ten mice were observed in each group, five for the primary response and five for the secondary response.
Table 5
Hemagglutination Titers of Mice Exposed to Aerosolized Lead

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Antibody and 95% Confidence Intervals</th>
<th>Beta-mercaptoethanol resistant (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-aerosolized Control:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary response</td>
<td>192 (164-282)</td>
<td>8 (6-8.5)</td>
</tr>
<tr>
<td>Non-aerosolized Control:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>secondary response</td>
<td>256 (172-289)</td>
<td>128 (92-165)</td>
</tr>
<tr>
<td>Two week Aerosolized Lead:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary response (30 µg Pb/day)</td>
<td>32a (22-40)</td>
<td>4 (3.5-5)</td>
</tr>
<tr>
<td>Aerosolized Lead:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>secondary response (80 µg Pb/day)</td>
<td>192 (163-229)</td>
<td>128 (91-143)</td>
</tr>
<tr>
<td>Chamber Born:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>primary response (80 µg Pb/day)</td>
<td>16a (15-18)</td>
<td>not performed</td>
</tr>
<tr>
<td>Chamber Born:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>secondary response (80 µg Pb/day)</td>
<td>64a (51-73)</td>
<td>32a (30-40)</td>
</tr>
</tbody>
</table>

a \( p < 0.01 \) as determined by AOV
b Ten mice were observed in each group, five for the primary response and five for the secondary response.
Pb<sub>ch</sub> compared to C<sub>air</sub> remained significantly suppressed in both total antibody and IgG antibody.

2.7 Complement Hemolytic Activity

The measurement of hemolytic complement activity is a widely used assay which serves as a convenient quantitative method to identify decreased complement activity.

2.7.1 In vitro Lead Exposure

The three lead compounds tested in the in vitro portion of the hemolytic complement assay were lead acetate, lead chloride and lead nitrate. All of the lead compounds were inhibitory on hemolytic complement activity. The degree of inhibition was dependent upon the concentration of lead added. The levels of inhibition (Table 6) are typical of the type of inhibition caused by a number of other heavy metals on complement activity. As we observed at levels > 100 µM, lead inhibition was produced up to the highest concentration of lead used. Inhibition values for lead nitrate at concentrations > 300 µM could not be obtained due to the lytic effects of higher concentrations of this compound on sheep erythrocytes.

For all compounds tested at concentrations less than 100 µM, no inhibition was observed.

To determine whether a competitive reaction existed between lead and calcium-magnesium, various concentrations of calcium-magnesium saline were added to the lead solutions prior to incubation with srbc. The final concentrations of calcium-magnesium used were 1000, 800, 500, 400 and 200 µM solutions. Inhibition of all lead compounds
Table 6
Inhibition of Complement Induced Hemolysis by Lead

<table>
<thead>
<tr>
<th>Lead Solution Used</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Lead Nitrate</strong></td>
<td></td>
</tr>
<tr>
<td>250 μM</td>
<td>75.4</td>
</tr>
<tr>
<td>200 μM</td>
<td>19.2</td>
</tr>
<tr>
<td>150 μM</td>
<td>1.5</td>
</tr>
<tr>
<td>100 μM</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>B. Lead Acetate</strong></td>
<td></td>
</tr>
<tr>
<td>400 μM</td>
<td>90.0</td>
</tr>
<tr>
<td>350 μM</td>
<td>89.0</td>
</tr>
<tr>
<td>300 μM</td>
<td>86.5</td>
</tr>
<tr>
<td>250 μM</td>
<td>13.1</td>
</tr>
<tr>
<td>200 μM</td>
<td>10.0</td>
</tr>
<tr>
<td>150 μM</td>
<td>4.4</td>
</tr>
<tr>
<td>100 μM</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>C. Lead Chloride</strong></td>
<td></td>
</tr>
<tr>
<td>400 μM</td>
<td>87.0</td>
</tr>
<tr>
<td>350 μM</td>
<td>85.5</td>
</tr>
<tr>
<td>300 μM</td>
<td>63.2</td>
</tr>
<tr>
<td>250 μM</td>
<td>27.5</td>
</tr>
<tr>
<td>200 μM</td>
<td>4.4</td>
</tr>
<tr>
<td>150 μM</td>
<td>2.5</td>
</tr>
<tr>
<td>100 μM</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a All solutions prepared in isotonic saline solution.
tested was reversed with the addition of a calcium-magnesium saline solution twice the molarity of the lead solution causing inhibition.

2.7.2 In vivo Lead Exposure

Results of the hemolytic C' activity in sera of mice exposed to aerosolized lead for two weeks are shown in Table 7, along with serum lead values. A 1:2 dilution of serum was used to measure the hemo-lytic complement levels of both a lead aerosolized group and a nonaeroso-lized control group. Activity of complement in serum was reduced to a highly significant degree (approximately 12%) in the lead exposed group compared to controls. The average serum lead content as determined by AAS for the two week lead aerosolized group (1.49 ppm) was ten times higher than that of the controls (0.147 ppm).

3. Biological Assays

3.1 Phagocytic Index

To determine the effects of lead exposure on the phagocytic ability of peritoneal and alveolar macrophage (MØ), a phagocytic index was determined on ingested and aerosolized lead groups and their respective controls.

3.1.1 Phagocytic Index of Orally Exposed Lead Groups

The phagocytic index (PI) of both peritoneal and alveolar MØ in group Pboral did not differ significantly from control group Coral. The number of alveolar and peritoneal MØ harvested from the orally exposed lead group was also similar to the number of phagocytes harvested from the control. Results of the PI and number of
Table 7
Hemolytic Complement Activity of Serum
After In Vivo Exposure to Aerosolized Pb

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Serum Lead Concentration</th>
<th>Average Percent Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Non-aerosolized)</td>
<td>0.144 ± 0.007 ppm</td>
<td>92.5 ± 5.3</td>
</tr>
<tr>
<td>Two Week Lead (Aerosolized)</td>
<td>1.485 ± 0.07 ppm*</td>
<td>80.5 ± 7.6</td>
</tr>
</tbody>
</table>

* p < 0.01
cells harvested are given in Table 8. With the results obtained thus far it appears that with lead ingestion at the subclinical levels used in this experiment, much of the lead entering the body is rapidly eliminated and excreted, thus allowing for normal phagocytic ability of both alveolar and phagocytic macrophage.

3.1.2 Phagocytic Index of Aerosolized Lead Group

The phagocytic index for the peritoneal \( M\) of groups \( Pb_{air}, Pb_{ch} \) and the control group \( Ca_{ir} \) is given in Table 8. Inhalation of lead at the quantity and duration as used in this experiment did not appear to effect the phagocytic ability of the peritoneal macrophage. The quantity of cells harvested from the lead exposed mice was also comparable to numbers of cells harvested from the respective controls. It appears therefore that the peritoneal \( M \) does not contribute to the immunosuppressive effect elicited by lead.

The major cellular effect of aerosolized lead appeared to be on the alveolar macrophage of both groups \( Pb_{air} \) and \( Pb_{ch} \). As the results in Table 9 indicate, a highly significant decrease in the PI was observed when compared to the control group \( Ca_{ir} \). The number of alveolar \( M \) recovered from both aerosolized lead groups was 3.5 times the number of cells recovered from the non-aerosolized controls.

3.2 \( LD_{50} \) Determination

Orally lead exposed mice and two week lead aerosolized mice along with controls for each group were challenged IP with \( Salmonella typhimurium \) to determine the \( LD_{50} \). The \( LD_{50} \) results are given in Table 10, along with the standard error and confidence
Table 8
Phagocytic Index of Peritoneal Macrophages Harvested By Saline Lavage in Lead Exposed Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Phagocytic Index</th>
<th>Harvested Cells (cells/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 0.66</td>
<td>1.45x10&lt;sup&gt;6&lt;/sup&gt; ± 1.5x10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Orally Fed Lead&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 ± 0.45</td>
<td>1.72x10&lt;sup&gt;6&lt;/sup&gt; ± 8.5x10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>(75 µg Pb/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two Week Lead Aerosolized&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2 ± 0.57</td>
<td>2.23x10&lt;sup&gt;6&lt;/sup&gt; ± 1.0x10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>(80 µg Pb/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chamber Born&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.7 ± 0.93</td>
<td>1.46x10&lt;sup&gt;6&lt;/sup&gt; ± 6.8x10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>(80 µg Pb/day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ten mice/group ± S.D.

<sup>b</sup> The number of fluorescent beads in ten macrophage per slide were counted to determine the phagocytic index.
<table>
<thead>
<tr>
<th>Group</th>
<th>Phagocytic Index ( \text{cells/ml} )</th>
<th>Harvested Cells ( \text{cells/ml} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group(^b)</td>
<td>6.1 ± 0.68</td>
<td>1.4x10^5 ± 4.7x10^4</td>
</tr>
<tr>
<td>Orally Fed Lead(^b) (75 µg Pb/day)</td>
<td>5.6 ± 0.63</td>
<td>2.3x10^5 ± 1.6x10^5</td>
</tr>
<tr>
<td>Two Week Lead Aerosolized(^b) (80 µg Pb/day)</td>
<td>4.3 ± 0.75(^c)</td>
<td>5.1x10^5 ± 1.4x10^5(^c)</td>
</tr>
<tr>
<td>Chamber Born(^b) (80 µg Pb/day)</td>
<td>4.5 ± 0.89(^c)</td>
<td>6.3x10^5 ± 1.4x10^5(^c)</td>
</tr>
</tbody>
</table>

\(^a\) Mean ten mice/group ± S.D.
\(^b\) Ten macrophage/mouse were counted for phagocytic index.
\(^c\) \( p < 0.01 \) as determined by one way AOV.
Table 10

Results of Lethal Dose 50
In Lead Exposed Mice
Using the Organism *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>Group</th>
<th>LD$_{50}$ and 95% Confidence Intervals$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control</td>
<td>3.3$\times$10$^5$ CFU/mouse</td>
</tr>
<tr>
<td></td>
<td>(4.3$\times$10$^4$ - 2.5$\times$10$^6$)</td>
</tr>
<tr>
<td>Orally Fed Lead (75 µg Pb/day)</td>
<td>1.89$\times$10$^5$ CFU/mouse</td>
</tr>
<tr>
<td></td>
<td>(2.3$\times$10$^4$ - 9.4$\times$10$^5$)</td>
</tr>
<tr>
<td>Non-aerosolized Control (80 µg Pb/day)</td>
<td>2.02$\times$10$^8$ CFU/mouse</td>
</tr>
<tr>
<td></td>
<td>(1.2$\times$10$^8$ - 8.0$\times$10$^8$)</td>
</tr>
<tr>
<td>Aerosolized Lead (80 µg Pb/day)</td>
<td>1.57$\times$10$^6$ CFU/mouse</td>
</tr>
<tr>
<td></td>
<td>(2.4$\times$10$^5$ - 9.9$\times$10$^6$)</td>
</tr>
</tbody>
</table>

$^a$ Irwin-Cheeseman (27) method used to determine 95% confidence levels.
intervals as determined using the method of Irwin and Cheeseman (27). In the ingested lead group, Pb<sub>oral</sub>, similar LD<sub>50</sub> values were obtained as for control group C<sub>oral</sub>, indicating no impairment of the immune system. In group Pb<sub>air</sub> a 128-fold difference in the LD<sub>50</sub> was seen when compared to the LD<sub>50</sub> for the nonaerosolized controls. It would appear that aerosolized lead does alter the immune system causing an immunosuppressive effect, when a gram negative organism is injected.

4. Chemical Methods

4.1 Atomic Absorption Spectrophotometry

Subclinical quantities of lead which are ingested are rapidly eliminated from the body, as reported previously. This appears to be the case in this study. Of the five organs measured for lead content, only the kidney indicated an increase in lead content compared to controls C<sub>oral</sub>. As a two-fold increase was observed in this group, these results compare favorably with results obtained by Koller et al. (33) who also reported increased kidney lead content with ingestion of subclinical amounts of lead in mice.

Inhalation of lead resulted in a greater retention with accumulation of lead in the kidney, liver, lung, thymus and serum. In group Pb<sub>air</sub>, the kidney lead content was raised nearly four fold over the control group C<sub>air</sub>. In addition, a highly significant increase in liver lead content and lung lead content was observed, as well as a significant increase in serum lead content when compared to the control group. Significant increases in the thymus, liver and lung
lead contents of group Pb_{ch} was observed compared to controls C_{air}. The spleen and blood lead levels did not differ significantly from controls in either of the aerosolized lead groups.
Table 11

Lead Concentrations in the Tissues and Blood
Of Mice Exposed to Ingested or Aerosolized Pb^c

<table>
<thead>
<tr>
<th>Group</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Liver</th>
<th>Lung</th>
<th>Kidney</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orally Fed Control</td>
<td>20.1 ± 7.9</td>
<td>16.2 ± 9.1</td>
<td>29.7 ± 11.3</td>
<td>9.7 ± 5.9</td>
<td>11.9 ± 4.6</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>Orally Fed Lead</td>
<td>14.3 ± 8.2</td>
<td>17.9 ± 12.4</td>
<td>31.0 ± 9.7</td>
<td>8.2 ± 1.5</td>
<td>21.5 ± 5.8^a</td>
<td>4.7 ± 2.4</td>
</tr>
<tr>
<td>Non-aerosolized Control</td>
<td>21.2 ± 10.0</td>
<td>14.8 ± 5.6</td>
<td>22.4 ± 5.4</td>
<td>6.2 ± 0.79</td>
<td>12.0 ± 5.0</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>Two Week Aerosolized Lead</td>
<td>32.2 ± 13.6</td>
<td>39.5 ± 18.8</td>
<td>63.3 ± 12.6^b</td>
<td>54.9 ± 35.8^a</td>
<td>43.8 ± 13.9^b</td>
<td>4.9 ± 3.8</td>
</tr>
<tr>
<td>Chamber Born</td>
<td>43.9 ± 16.2^a</td>
<td>43.1 ± 28.5</td>
<td>53.9 ± 23.0^a</td>
<td>101.8 ± 66.0^a</td>
<td>50.4 ± 12.2^b</td>
<td>5.5 ± 2.5</td>
</tr>
</tbody>
</table>

^a p < 0.05 as determined by the Analysis of Variance method
^b p < 0.01 as determined by the Analysis of Variance method
^c Mean of 10 mice ± S.D.
^d Results reported as parts per million lead in organ dry weights
DISCUSSION

The effects of toxic substances have been evaluated by parameters such as carcinogenicity, teratogenicity, embryogenicity and neurotoxicity. The study of alterations in immunocompetence by toxic substances has been greatly neglected in the past. This oversight may pose a serious hazard since toxic substances can impair the capacity for mounting immunological reactions which seriously endangers the health and life span of both man and animal. Recently the immunosuppressive effects of lead exposure have been assessed using criteria such as those in this study.

1. Effects of Lead on the Immune and Hematopoietic Systems

1.1 Hematocrit

Anemia is one of the first clinical indications of lead toxicity. Although it is known that anemia occurs with severe lead intoxication, the threshold blood lead level at which anemia occurs has not been clearly established (18). Hematocrit levels as determined in this study are used as an indicator of anemia.

Hematocrit levels of the lead administered groups were not significantly different from those of control animals. This result suggests that a subclinical level of lead was administered in this study.

1.2 Organ to Body Weight Ratios

Organ to body weight ratios were calculated for lymphoid organs to determine changes produced by lead exposure. Animals
administered lead orally had no statistically significant differences in organ to body weight ratios compared with control animals. This finding is not unusual since 1) the concentration of lead used was of a subclinical level and 2) a large portion of ingested lead (~90%) is rapidly excreted from the body (29).

Animals in the lead inhalation group had significant weight changes in a number of organs. Animals exposed for two weeks, Pbair, had a highly significant (p < 0.01) decrease in the thymus to body weight ratio. This decrease was not observed in the chamber born Pbch group, although a 21% decrease in thymus weight did occur. Whether the observed thymic atrophy associated with these groups resulted directly or indirectly in a T-cell dysfunction (31,32) was not assessed.

The spleen to body weight ratio in both aerosolized lead groups was decreased significantly (p < 0.01). The spleen is associated with the humoral or antibody mediated immune response, therefore, this decrease in organ weight could be associated with reported impairment in B-cell immunity (31,32,33,35).

Hepatic atrophy occurred only in the chamber born group (p < 0.01). The liver serves as a detoxification organ containing a number of enzymes that are required for endotoxin neutralization which might account for the enhanced susceptibility previously observed (52).

Kidney to body weight ratios in both group Pbair and Pbch increased to a highly significant degree compared with control group Cair. Similar increases in kidney weights have been reported by a
number of investigators (34). The kidney is also an essential filtrate organ containing numerous enzymes used in neutralization.

The lung to body weight ratio remained normal in both aerosolized groups compared with controls.

1.3 Total and Differential Leukocyte Counts

The cytotoxic nature of lead has been clearly demonstrated by a number of investigators (40) who have shown that extremely low levels of lead (10^{-5} M) can cause cellular death. This toxicity appears to involve lysosomes, inclusion bodies and reduced cellular enzymatic activity.

Alterations in leukocytes may lead to impaired humoral as well as cellular immune responses, therefore, total and differential leukocyte counts were performed to determine any changes in these parameters.

Differential counts as well as total leukocyte counts remained unaffected in the orally administered lead group compared with controls. Again this is further evidence that a large proportion of the ingested lead is excreted prior to entering the circulatory system.

Both aerosolized lead groups, Pb_{air} and Pb_{ch}, had significant changes in total and differential leukocyte counts. A significant neutrophilic shift to lymphocytes was observed in both groups. Group Pb_{air} had a significant (p < 0.05) increase in lymphocytes and a highly significant (p < 0.01) decrease in neutrophils. Group Pb_{ch} had a significant (p < 0.05) increase in lymphocytes and a significant decrease in monocytes. Both monocytes and neutrophils serve as phagocytic cells, and a decrease in both cell types may lead to lowered bacterial clearance and decreased cellular immunity.
1.4 **Cellulose Acetate Electrophoresis**

Through cellulose acetate electrophoresis the major globulins found in serum can be separated and quantitated to determine changes in these globulin fractions. The serum globulins are separated into four basic groups; albumin, alpha globulins, beta globulins and gamma globulins.

Sera obtained from the orally lead administered group indicated no abnormalities in globulin fractions compared with control sera, whereas both aerosolized lead groups had reduced globulin fractions. The gamma globulin fraction which contains the majority of immunoglobulins was reduced by 24% in group Pbair and by 36% in group Pbch. In addition, the beta globulin fraction containing complement associated proteins and blood clotting factors was reduced to a highly significant level in group Pbch (>40% reduction). The albumin level in this group increased 17% to compensate for the reductions in gamma and beta globulin levels.

A reduction in antibody levels as well as immunoglobulin synthesis with lead exposure have been previously reported (8,16,18,25,31-38, 42). A major finding thus far in this study is that significant immunosuppression has been established with exposure to lead at levels considered subclinical and in quantities approaching levels found in urban centers.

1.5 **Direct Hemagglutination**

To measure the overall competence of the immune system a direct hemagglutination assay was performed on experimental and control animals. The direct hemagglutination assays both T and B cell
function and combines high degrees of sensitivity and specificity using sheep red blood cells as the antigen.

Titers for group Pb_{oral} were comparable to control C_{oral}. These results were in agreement with the serum profiles obtained for this group discussed in the previous section.

Both aerosolized groups (Pb_{air} and Pb_{ch}) had highly significantly lowered primary antibody responses for IgM (19S) antibody compared with control C_{air}.

The secondary response of the two week lead aerosolized group was comparable to that of the control group, whereas the secondary antibody response in group Pb_{ch} remained significantly (p < 0.01) suppressed.

These data correlate with the results of the electrophoretic serum profiles which indicated reduced gamma globulin fractions. Whether impairment in antibody synthesis is due to dysfunction in the T-cell or B-cell populations was not established. Koller and Kovacic (35), who reported lowered antibody response to srbc in mice exposed to 1300 ppm lead acetate administered orally, suggested the specific impairment was due to T-helper cell dysfunction. This fact as yet has not been conclusively established.

1.6 Complement Hemolytic Activity

Complement refers to a group of interacting serum proteins, many of which are capable of enzymatic activity. Complement was originally detected by its ability to cause cytolysis of antibody sensitized (bound) bacteria and erythrocytes. It is now clear that
complement mediates a number of additional reactivities other than cytolysis, such as opsonization and chemotaxis (43,44).

Eleven complement proteins have been identified in the usual antibody initiated hemolysis; these comprise the classical pathway. A number of these components require calcium for their integrity and for reactivity. The most calcium dependent is the Cl complex, consisting of three proteins, Clq,Clr and Cls, linked together by calcium (10).

1.6.1 In vitro lead exposure

Our data on the effects of three lead compounds on complement activity indicated that lead at concentrations >100 μM produced a profound inhibitory effect on hemolysis. The amount of inhibition to hemolytic complement activity was concentration dependent with the greatest inhibition observed at the highest lead concentration (>300 μM). Lead nitrate levels >300 μM caused a lytic effect and therefore inhibition could not be determined. At lead concentrations <100 μM no noticeable inhibitory effects occurred. These results are comparable to those obtained for other heavy metals which produced inhibition of hemolytic complement activity (43,44).

This inhibitory effect was found to be reversible with the addition of Ca-Mg saline at twice the concentration of the lead solution which caused inhibition. It is therefore apparent that lead interferes with the binding of components either by 1) causing a separation of the Cl complex through replacement of Ca by Pb, 2) replacing calcium at receptor sites which may require calcium, or 3) replacing calcium required for the enzymatic action of specific complement components.
1.6.2 In vivo lead exposure

Hemolytic complement activity in mice exposed to aerosolized lead for a two week period was inhibited 12% compared with normal non-aerosolized controls. These results are similar to those obtained by Montgomery et al. (44) in which zinc glycine injected intravenously in rabbits at two to three times the physiological serum zinc concentrations resulted in significant inhibition of total C' activity in sera of treated rabbits.

Lead serum levels determined by atomic absorption in group Pbair mice revealed a 10-fold increase in Pb compared to nonexposed controls. This is unusual since the majority of lead was found to be contained in serum rather than in the blood clot containing the red blood cell portion.

2. Biological Assays

2.1 Phagocytic Index

Phagocytosis serves a critical role in the clearance of foreign substances. Bacteria, viruses and foreign particles are readily eliminated by specific phagocytic cells, generally macrophages (MØ). Three major groups of MØ exist, the alveolar macrophage found in the lungs, the Kupffer cells found in the liver and the peritoneal macrophages located in the peritoneum. The phagocytic activity of the alveolar and peritoneal macrophages were measured in this study.

Lead administered to mice via gastric intubation caused no impairment of phagocytic activity in either alveolar or peritoneal macrophage. The number of MØ harvested from each area was equivalent to
the number harvested from control group \(C_{\text{oral}}\). These data are not unusual in that other parameters measured up to this point for group \(P_{\text{oral}}\) have resulted in no statistically significant changes, indicating rapid clearance of ingested lead.

In both aerosolized lead groups (\(P_{\text{ch}}\) and \(P_{\text{air}}\)) the phagocytic index (PI) and number of \(M\theta\) harvested from the peritoneal cavity were equivalent to those obtained from control group \(C_{\text{air}}\).

A 21% decrease in the PI of alveolar \(M\theta\) resulted in groups \(P_{\text{air}}\) and \(P_{\text{ch}}\), whereas, the number of alveolar \(M\theta\) collected from these groups was 3.5 times the number harvested from group \(C_{\text{air}}\). Impaired phagocytic ability was anticipated in this group for the following reasons: 1) lead suppressed humoral antibody formation; 2) lead nitrate injected intraperitoneally impaired Kupffer cell phagocytosis (54); and 3) bacterial clearance was impaired following lead instillation (7). The increase in the number of cells harvested from the lungs was an unusual finding in that a number of investigators have clearly demonstrated the cytotoxic property of lead and other heavy metals on \(M\theta\) (6,7,11,28,36,45).

It appears that much of the inhaled lead enters the pulmonary system and subsequently inhibits the phagocytic activity. Whether this inhibition is due to impaired enzymatic activity produced by lead as suggested by Kaminski (28) or whether lead produces a proliferation of immature phagocytes as Bouley (7) suggests has not been determined. Although a large quantity of lead enters the hematopoietic system with inhalation, at the concentration used it is evident that lead does not reach the peritoneal \(M\theta\) or that these phagocytes are less sensitive to the cytotoxic effect of lead.
2.2 LD$_{50}$ Determination

Decreased immunologic reactivity at both the humoral and cellular levels has been shown to occur with lead exposure. To determine whether acquired immunity is impaired in vivo following lead exposure as reported by Hoffman (26), Hemphill (25) and Selye (52), LD$_{50}$ determinations using *Salmonella typhimurium* were performed.

Results for the LD$_{50}$ obtained for group Pboral were similar to those obtained for controls Coral. This finding correlates with previous results for this group. Dysfunction of the immune system was not evident in this group suggesting lead ingested at the quantity used in this group was rapidly excreted and eliminated.

Inhalation of lead resulted in very large differences in the LD$_{50}$ value. A 128-fold decrease in LD$_{50}$ titer occurred in group Pbair. Possible factors responsible for this decrease are the following:

1) antibody levels were reduced; 2) phagocytic activity of specific MØ was inhibited; 3) complement activity was reduced; or 4) liver enzyme function was impaired resulting in decreased endotoxin neutralization (16,26). All of the previously mentioned factors alone or together can easily account for the increased mortality seen in the lead aerosolized group. These results further demonstrate our observations that inhaled lead poses a greater health hazard than previously determined.

2.3 Lead Concentration in Tissues

A rapid elimination of lead occurs after ingestion, as is evident from atomic absorption spectrums obtained from various tissue
samples. Only the kidney had elevated \( p < 0.05 \) lead levels in group \( \text{Pb}_{\text{oral}} \) compared with controls. This is not an unusual finding; Koller et al. (33) reported elevated kidney lead levels in mice administered lead orally. It is interesting to note that no impairment was observed for any of the parameters measured in this group; in addition, lead levels in blood, serum, liver, lung, spleen and thymus all were of a normal level, again indicating rapid elimination of ingested lead.

Inhalation of lead resulted in elevated lead tissue levels in all organs with the exception of the blood and spleen. This is unusual because antibody formation (due to B-lymphocytes found in the spleen) was decreased. The findings that lead levels in the spleen were normal and normal peritoneal \( \text{M}^\circ \) function was observed suggest that circulating lead may not reach this area to any great extent. If this is the case, the B-lymphocyte may not be responsible for the reduction in antibody to srbc and \textit{Salmonella typhimurium}. The T-lymphocyte may be the predominant cell type affected by lead. These data also indicate the need for improved monitoring of lead levels other than blood levels, since these were found to be comparable to controls. Similar findings were reported by Schlick (48) who reported normal blood lead levels in exposed animals. Since serum lead levels were found to be greatly elevated (10\%) in the aerosolized lead group, monitoring of sera rather than whole blood may prove a more sensitive indicator of lead exposure.
Summary

At the subclinical dose of lead administered in this investigation it has become apparent that the route of exposure plays a critical role in the immunotoxic effects produced by this element. Ingested lead resulted in little or no adverse immunotoxic effects. The same quantity of lead, when inhaled resulted in suppression of both cell mediated and humoral immune responses. Observed cellular effects included a reduction of monocytes, decreased phagocytic activity of alveolar macrophage and decreases in neutrophils leading to an increase in the lymphocyte population. Humoral suppressive effects seen were decreased antibody levels, increased susceptibility to bacterial infection, lowered antibody titers to T and B cell immunogen and decreased hemolytic complement activity, both in vivo and in vitro. Other observed effects included elevated lead levels in liver, lung, spleen, thymus and serum. These changes all occurred without indication of anemia (normal hematocrit), suggesting subclinical exposure to lead.

It was also observed that blood lead levels (clotted blood) were not elevated from any of the exposed groups using atomic absorption spectrophotometry. Similar results have previously been reported (48). This finding further demonstrates the need for a more sensitive method for determining exposure to lead compounds.

With the results reported in this study it has been demonstrated that lead exposure poses a significant health hazard which could lead to lowered ability to resist infection, as well as lower the immunosurveillance mechanism thereby increasing the chance of neoplasia.


Appendix

Trypan Blue Dye Solution (Stock)

A. Trypan Blue Dye Solution (Stock)

- Trypan Blue: 1 g
- Distilled Water: 100 ml

(Working)
- Trypan Blue (Stock): 1 ml
- Isotonic Saline: 9 ml

B. Bouins Fixative

- Picric Acid, saturated aqueous: 75 ml
- Formalin: 25 ml
- Glacial acetic acid: 5 ml