IOWA STATE UNIVERSITY
Digital Repository

1977

Physiologic and hematologic studies on Mycoplasma hyopneumoniae pneumonia of swine

Yanyong Intraraksa
Iowa State University

INFORMATION TO USERS

77-25,994

INTRARAKSA, D.V.M., Yanyong, 1940-
PHYSIOLOGIC AND HEMATOLOGIC STUDIES ON
MYCOPLASMA HYOPNEUMONIAE PNEUMONIA OF SWINE.

Iowa State University, Ph.D., 1977
Veterinary Science

Physiologic and hematologic studies on <u>Mycoplasma</u>

<u>hyopneumoniae</u> pneumonia of swine

by

Yanyong Intraraksa

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Veterinary Anatomy, Pharmacology
             and Physiology
     Major: Physiology

Approved:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1977

TABLE OF CONTENTS

INTRODUCTION

In most countries where pigs are agriculturally important, reports on industrial problems and research indicate that respiratory disease is an economically significant problem. The most common respiratory disease of pigs is mycoplasmal pneumonia. It has been estimated that 40-50 percent of all pigs are affected with mycoplasmal pneumonia (Switzer, 1969). Mycoplasmal pneumonia of pigs has been responsible for substantial economic loss, and has a worldwide distribution. Mycoplasma hyopneumoniae was shown to be the causal agent (Maré and Switzer, 1965). The chronic mycoplasmal pneumonia occurs mostly in young pigs between 3 and 10 weeks of age. There are many workers that have already reported the clinical signs and pathologic lesions, with complete details of the disease. Mycoplasmal pneumonia is described as a chronic, low mortality, high morbidity disease of swine characterized by coughing, loss of vigor, growth retardation, and decreased efficiency of food utilization. Livingston et al. (1972) completely described the histopathological change of the lung post inoculation of M. hyopneumoniae. They noted the first change was small lymphoid nodules extending through the alveolar area. Gross lesions became evident at the 14th day post infection and consisted of lymphocytic cells and inflammatory exudate within bronchi and alveoli. The greatest severity of the microscopic lesions occurred between 22 to 38 days and included lymphoid hyperplasia and severe damage of alveoli. The severity of the microscopic lesion is mild after 42nd day post infection. Livingston and co-workers also reported the loss of cilia over the bronchial epithelial cells. The extensive microscopic damage of the

alveolar area by M. hyopneumoniae could lead to the physiological alteration of the respiratory system.

The lung is a vital organ for gas exchange. Many workers have studied the nature of the interface between air and alveolar tissue. Clements et al. (1961) described the surface phenomena in relation to pulmonary function and showed the importance of the film lining the alveolar surface. Mammalian lungs contain a specialized acellular lining material with surface active properties that stabilize the pulmonary alveoli against collapse (Clements et al., 1958). A film of this surfactant material has characteristic properties in which the dynamic surface tension decreases sharply as the surface area is decreased and increases sharply as the surface area is increased. The phospholipids are the major component of the pulmonary surfactant secreted from the type II alveolar epithelial cells as demonstrated by Macklin (1954), Scarpelli (1968b), and Heinemann and Fishman (1969). There are several pathological conditions that alter the secretion of pulmonary surfactant and subsequently the gas exchange.

This study was undertaken to measure physiological and hematological changes in the pigs with induced mycoplasmal pneumonia. Two experiments were conducted. The first experiment was designed to study the changes of respiratory parameters such as volume, expansibility (elasticity), ventilatory ability (inspiratory and expiratory velocity), and the arterial blood gas measurements on the 4th, 5th and 6th week post inoculation of M. hyopneumoniae. Studies of hematological changes were made in order to assess the pathologic condition of the disease. The pulmonary

surfactant was collected by lung lavage for chemical analysis. The second experiment was designed to study the phospholipid synthesis at the 4th and 5th week post infection by intravenous injection of palmitate-^{14}C and to determine the radioactivity in the lung as well as in pulmonary surfactant.

In addition, the pigs are now considered to be useful as an animal model for research in human respiratory disease because pigs have submucosal glands which compare closely in degree of development and distribution with those of man (Goco et al., 1963). Since the young children also suffered from pneumonia which is caused by a virulent Mycoplasma pneumoniae, it is possible that the information on the physiological and hematological changes in mycoplasma pneumonia of pigs could be used for comparative studies in man.

REVIEW OF LITERATURE

The Mycoplasmal Pneumonia of Swine

The disease has been referred to as swine enzootic pneumonia (SEP) or virus pneumonia of pigs (VPP) and has been described as the most wide-spread and economically important swine disease in the world (Betts, 1952; Young, 1964). The prevalence of mycoplasmal pneumonia of swine has been estimated to vary from 34 to 74 percent (Betts, 1952; Young and Underdahl, 1955; Young and Underdahl, 1960). The disease has no special seasonal occurrence.

Maré and Switzer (1965) and Goodwin et al. (1965) demonstrated that the causative agent of this disease in not viral but a mycoplasmal organism. Maré and Switzer (1965) reported the isolation and propagation in cell-free fluid and solid mediums of an agent capable of eliciting the pneumonia in 80 to 100 percent of the inoculated respiratory disease-free pigs. They showed that the causative agent of the disease was a Mycoplasma sp. and named it Mycoplasma hyopneumoniae. This finding has been confirmed in Canada by L'Ecuyer (1969), in Japan by Takatori (1969), in England by Roberts (1968) and by Hodges et al. (1969).

Goodwin et al. (1965) were able to grow a mycoplasma which induced a enzootic pneumonia. They named it Mycoplasma suipneumoniae (Goodwin et al., 1967). However, because the cultures of M. hyopneumoniae and M. suipneumoniae were indistinguishable by the growth inhibition and metabolic inhibition test, Edward and Freundt (1969) suggested that the species designation of hyopneumoniae has priority over suipneumoniae and

should be used to designate the causative agent of the chronic mycoplasmal pneumonia of swine. Maré (1965) estimated that the organism is between 110 mu and 225 mu in diameter. Observation of Giemsa stained preparation revealed coccoid and cocco-bacillary bodies with occasional ring forms.

Mycoplasmal pneumonia is spread from one pig to another by direct contact or by inhalation of airborne Mycoplasma. Young pigs usually contact the pneumonia from their mothers (Young, 1964). At the present time there is no effective treatment for mycoplasmal pneumonia of swine. Several of the broad spectrum antibiotics such as chlortetracycline and oxytetracycline will suppress development of the pneumonic lesion if the animal is receiving a drug at the time of exposure. Sulfonamides, penicillin, and streptomycin have been reported as useless in treatment by Betts (1952).

Maré (1965) and Livingston et al. (1972) studied the details of the macroscopic lesions of mycoplasmal pneumonia. Histologic changes were found in the lungs on the 6th post inoculation day. The gross lesion can be found in the lung of infected pigs at the 14th day post inoculation. The lesions are found predominantly in the apical, cardiac, and intermediate lobes of the lung, and the left cardiac and left apical lobes are affected less frequently. The frequency and location of lesions in the different lobes of the lungs can be attributed to the mechanical barrier produced by the walls of the trachea and bronchi. The gross lesions developed to maximal severity between the 21st and 28th days and then they regressed. The mycoplasmal pneumonia is histologically characterized by clearly demarcated, reddish-blue to light pinkish-grey colored lesions in

the affected lobes. Microscopic lesions consist of alveolar interstitial thickening, giant cell formation, neutrophil infiltration, septal cell proliferation, and peribronchiolar and perivascular lymphoid hyperplasia. Pattison (1956) and Jericho (1968) suggested that there are several agents that can result in grossly similar lesions.

The humoral immunity

Lannek and Bornfors (1957) showed that a strong immunity developed in experimentally induced pneumonia. Pigs were infected, allowed to recover and then challenged 118 days after the primary infection. All the challenged pigs were free from enzootic pneumonia at slaughter, whereas all of the positive controls had pneumonia.

Roberts (1968) was able to detect antibodies to M. hyopneumoniae in pig serum by using the standard complement fixation (CF) test. Boulanger and L'Ecuyer (1968) also demonstrated antibodies to the organism at two to three weeks after experimental injection by the use of a modified direct complement fixation test. The antibodies remained detectable for more than 200 days. Boulanger and L'Ecuyer were also able to demonstrate antibodies by an indirect CF test but the titer obtained was lower than that measured with the modified test. Takatori (1969) also demonstrated CF antibodies in pigs experimentally infected with M. hyopneumoniae. He also reported that anitbodies first appeared two to three weeks after inoculation. Goodwin et al. (1969) detected CF antibodies in pigs infected with M. hyopneumoniae and found no correlation between either the extent of the pneumonic lesions or the immune state and the titer obtained. They also successfully employed an indirect hemagglutination test for the

detection of antibodies against M. hyopneumoniae. They also found that pigs which had recovered from the disease were strongly immune to challenge and no lung lesions developed when the animals were inoculated with lung suspensions that produced extensive lesions of enzootic pneumonia in control animals.

Lam and Switzer (1971) developed an indirect hemagglutination (IHA) test for the detection of antibodies to M. hyopneumoniae in swine. They showed that in serum of pigs with naturally occurring infection antibodies were detected in 68 to 87 (78.1%) pigs having macroscopic pneumonic lesions and in 56 of 82 (68.2%) pigs not having lesions. They did a serologic test of 433 swine serum samples from various field herds, and found antibodies against M. hyopneumoniae. Two hundred ninety-one of the sera were positive (67.2%). Lam (1970) showed that by using an indirect hemagglutination (IHA) test for the detection of M. hyopneumoniae infection, pigs injected with the agent developed antibodies two to three weeks post infection and maintained that level for 130 days. The level of antibody started to fall off gradually after that, but remained detectable for at least 320 days post-infection. He also reported that the healthy pigs in contact with pigs that had just been injected with live culture of M. hyopneumoniae developed antibody titers in five to six weeks. When susceptible pigs were in contact with pigs that had been injected two months previously, the susceptible pigs developed antibody in seven to eight weeks but failed to develop lung lesions.

At the present time the complement-fixation test is the best technique available for the epidemiologic investigation of mycoplasmal

pneumonia of swine. However, the conventional complement-fixation test for M. hyopneumoniae antibodies, considered satisfactory as a herd test, is not reliable for the serum samples from individual pigs. The older pigs inoculated with M. hyopneumoniae did not produce a positive M. hyopneumoniae complement fixation reaction as reported by Robert and Little (1970). Slavik and Switzer (1972) modified the method of micro-titration complement-fixation. This method can detect the antibody of experimentally infected pigs as early as two weeks post-infection and as long as 24 weeks post-infection. The sensitivity of the CF test was increased greatly by heating the test sera at 56°C for 30 minutes and by using normal, unheated swine serum as the reconstituting fluid for the lyophilized guinea pig complement. Slavik and Switzer reported that this technique can detect antibodies to M. hyopneumoniae at dilution of test sera as low as 1:8 and no complement-fixation cross-reactions were observed among other mycoplasmal species. Switzer and Preston (1974) used this micro-complement-fixation test for the large number of pigs with a wide range of age in Iowa. They found that the highest percent of infected pigs were pigs less than one year of age.

Robert (1973) reported observations on the cell-mediated immune response to M. hyopneumoniae, as measured by macrophage migration inhibition, the pigs infected with the organism also gave a positive intradermal reaction within 24 hours following inoculation. However, the control and infected pigs used in both the intradermal and macrophage migration inhibition tests were in small number. These methods could be used as a reliable diagnostic aid but require more research.

The Surfactant Systems of the Lung

The term "surfactant" refers to a substance which is surface active. The phrase "pulmonary surfactant" is used to denote the substance responsible for the unusually great surface activity present in mammalian lungs. There are many physiological studies indicating that extremely low surface tension (i.e., 10 dynes/cm or lower) must be present within the air-filled lung when it is at a low volume. It is observed that a lung does not collapse completely when very low distending pressures are present. Alveolar surface tension, especially at low lung volumes, would become high and the alveoli would be apt to collapse unless the surface forces of the air-tissue interface could be stabilized. In 1929, Von Neergard was the first to postulate that a stabilizing substance must be present in the lung. He demonstrated the effects of surface forces on the pressure-volume characteristics of excised lung. Macklin (1954) reported that the mucoid film on alveolar wall performs vital functions, such as assisting in the removal of fine lining or dead particulate matters, the maintenance of a constant favorable alveolar surface tension, the facilitation of gaseous exchange, the protection of the underlying tissue from desiccation, and the suppression of invading microorganisms. He also suggested that the film of the alveolar wall is secreted by granular pneumocytes. The preliminary studies showed that the alveolar lung fluid forms an insoluble film at an air-liquid interface and modifies the surface tension of this interface in a manner which depends on alveolar surface area (Pattle, 1958; Clements et al., 1958; Avery and Mead, 1959). Clements et al. (1958) showed the effects of surface tension on lung mechanics and postu-

lated that this interfacial material would reduce the pressure acting across the alveolar interface and thereby prevent the collapse of the easily distensible alveolar units. In the higher mammals, the numerous alveoli provide the air-liquid interface of very large surface area. Scarpelli (1968b) has termed this material, because of its complex chemical nature, "the pulmonary surfactant system."

The cellular origin of pulmonary surfactant

There are two types of cells occurring on the alveolar side of the basement membrane: Type I alveolar epithelial cells are very thin averaging 0.5 μ in thickness and type II alveolar cells are round and large ranging from <10 to 15 μ in thickness. The alveolar surface of the epithelial cells particularly the surface of the type II cell that possesses microvilli, is rough. There are two layers of the acellular materials as shown by functional difference. The outside film facing the alveolar air is composed of densely spaced, highly surface-active phospholipids which are recruited from the underlying hypophase during expansion of the lungs and may reenter the hypophase at low lung volumes as described by Pattle (1965). The second layer is the base layer or the hypophase as described by Scarpelli (1968b). Electron microscopic and histologic studies by Bensch et al. (1964) and Kikkawa et al. (1965) have demonstrated that the inclusion bodies of type II cells are secretory. Naden (1967) reported that the inclusion bodies of the type II alveolar cell contain a surface active substance and are removed from the alveolar surface by phagocytosis. He proposed that the nonciliated, or Clara cells of the bronchioles synthesize the surfactant. His evidence was histochemical

studies which showed the phospholipid in Clara cells and autoradiographs which were interpreted as showing localization of primary precursor in Clara cells as soon as 5 minutes after injection.

Autoradiographs by Buckingham et al. (1966) used tritium labeled palmitate and acetate. They found labeled grains over the cytoplasm of granular pneumocytes near but not over the inclusion vacuoles. Askin and Kuhn (1971) injected tritiated palmitate into rats. By autoradiography and thin layer chromatography they found that there was progressive incorporation of labeled fatty acid into lecithin from 5 minutes to 4 hours after injection with much less incorporation into other lipid classes. Autoradiographs showed consistently heavier labeling of alveolar cells than of Clara cells. Electron microscopy demonstrated that the label was localized in the cytoplasm of granular pneumocites and was most concentrated over the osmiophillic inclusions. This experiment indicated that granular pneumocytes are a major source of alveolar lecithin. Massaro and Massaro (1972) incubated the amino acid leucine-4, 5-^{3}H with rat lung slices and used morphometric methods to determine the location of ^{3}H. The lungs that were removed 4 minutes after the addition of leucine showed 70% of the grains in type II cells to be above the rough endoplasmic reticulum and 10% to be over the lamellar bodies. By 40 minutes after the injection the relationship had changed so that 56% of the grains were over the rough endoplasmic reticulum and 29% over the lamellar bodies. Chevalier and Collett (1972) reported similar studies with mouse lung but they used three potential precursors of the surfactant: choline-^{3}H, leucine-^{3}H, and galactose-^{3}H. Between 30 and 120 minutes after injection of any of the three precursors, the number of grains over the lamellar

bodies increased relative to the number of grains elsewhere. According to Page-Roberts (1972) the lamellar bodies contain a high ratio of phospholipid to protein and the phospholipid is the surface active material. Kikkawa et al. (1975) used a method for isolation of type II alveolar epithelial cells which yield 95% purity. By using radiolabeled studies they showed that the isolated type II cells synthesized disaturated lecithin predominantly through the cytidine diphosphocholine pathway. The ^{14}C-choline incorporation into disaturated lecithin was three times as active in type II cells as in macrophages. The rate of surfactant metabolism is rapid, as calculated from studies with radioactive precursors of dipalmitoyl lecithin (Tierney et al., 1967). They found that the biological half-life of radioactivity was approximately 14 hours in rat. They also pointed out that the short half-life of saturated lecithin is concordant with the rate of turnover of surface-active alveolar lining calculated from observations of alveolar instability. Pawlowski et al. (1971) injected ^{3}H-palmitate dissolved alcohol into dogs and then removed lungs at intervals during the next 6 hours. They extracted their surface-active fraction from both the lavage material and from lung. Their results showed the half-life in the lung is 5 hours, and the specific activity of phosphatidyl choline in the lavage material did not reach that activity in the residual lung. Young and Tierney (1972) injected palmitate-1-^{14}C into rats, and determined the specific activity of dipalmitoyl phosphatidyl lecithin (DPL) in the lung tissue and in the fluid removed by a lung lavage. They found that the specific activities of DPL in the lung tissue and lavage fluid did not demonstrate a simple precursor-product type of relationship, since the DPL in lavage fluid has

a higher specific activity at 8 hours but not at 48 and 72 hours after the injection, and the decreasing specific activity of DPL in lung tissue did not follow a single exponential change with time.

Chemical composition of pulmonary surfactant

There are many techniques for determining the composition of the surfactant. The lavage technique and centrifugation are currently used for surfactant separation. The utilization of isolated cell population enriched in type II alveolar cells (Kikkawa et al., 1975) and fractionated by the methods of Gil and Reiss (1973) may ultimately provide the most unambiguous results.

Galdston et al. (1969) isolated a highly surface active fraction in the sediment of cell-free lung wash of excised rabbit lung by a simple centrifugation technique. They found that the sediment consists of about 60% lipid (lecithin) and 40% protein by weight. Of the lipid, about 90% is phospholipid and 10% nonphospholipid. By gas-liquid chromatography, the total fatty acids of the lipid moiety were found to be equally distributed among saturated and unsaturated acids. Frosolono et al. (1970) studied the chemistry of a surface-active fraction from dog lung. They found that the fraction contained lipid, protein, and carbohydrate, and the predominant lipid present was dipalmitoyl phosphatidyl choline. In 1971 Pfleger and Thomas studied the lipid composition of the surfactant and the exfoliated lining cells in normal beagle dog lungs. They found that phospholipids accounted for more than 70% of the surfactant and phosphatidyl choline was the predominant phosphatide. Phosphatidyl glycerol is present in beagle dog pulmonary surfactant (10% of Phospholipid), in exfoliated lining cells (7% of phospholipid), and in lung

tissue (3% of phospholipid). Body (1971) reported the phospholipid com-
position of pig lung surfactant, and found that pulmonary surfactant
contained 80% lipid material, 1.5% phosphatidyl glycerol, 2.0% lyso-bis-
phosphatidic acid.

Pfleger et al. (1972) isolated and characterized a phosphatidyl
glycerol from pulmonary surfactant lipids of beagle dogs. They reported
that phosphatidyl glycerol accounts for 10% of the surfactant phospholipid
and was at least as surface active as lecithin. King (1974) classified
the lipid composition of surface-active fractions obtained from the lung
into three groups: a) unfractionated lung and lavage fluid (Group A), b)
fractions isolated by relatively simple techniques, generally employing
one or two steps of centrifugation of physiological saline (Group B, and
c) fraction isolated by more involved techniques including differential
centrifugation (Group C). Abrams (1966) isolated the lipoprotein from the
homogenates of mammalian lungs. This lipoprotein can lower surface tens-
sion to 8 dynes/cm or less at a surface area of 20 cm^2. He also reported
that the lung of babies who died of hyaline membrane disease contained
less than 1/10th the amount of surface-active lipoprotein in normal infant
lung. Clements (1970) pointed out that the composition and the physiolog-
ical importance of the protein found in surface-active material have been
difficult to assess. The lipoprotein isolated from rabbit lung wash was
shown by immunologic techniques to have come from lung and not from blood
(Galdston et al., 1969). Galdston and Shah (1970) reported that the
association between lipid and protein appears to be of the Van der Walls
type, and lipoprotein showed the high surface properties. However,
Scarpelli et al. (1967) analyzed the pulmonary surfactant from dog and

rabbit lung and observed no lipoprotein complex. By using several methods for surfactant extraction such as Abrams (1966), Klein and Margolis (1968) and Galdston et al. (1969), Scarpelli et al. (1971) proved that the surfactant was not a lipoprotein complex. Klein and Margolis (1968) purified the pulmonary surfactant from rabbit lung by ultracentrifugation. They found that the surface active materials include protein. Colacicco et al. (1973) studied the protein in the lavage from rabbit lungs. They separated the pulmonary washing from rabbit lungs into four fractions by gel filtration through Sephadex G-200. The void volume fraction contains 87% of the phospholipid and most of two proteins designated S and T. These proteins can be separated from each other by centrifugation at 49,000 x g for 1 hour. The protein S together with over 60% of the phospholipid, is found in the pellet while protein T is found in the supernatant. King et al. (1973) isolated the protein components of canine surface active material obtained by lung lavage. They identified three electrophoretically and immunologically distinct proteins. Two proteins with molecular weights of about 11,000 and 34,000 daltons comprise most of the material with a relatively small amount of a 69,000 dalton protein. Immunologic analyses suggest that the 69,000 daltons component is albumin, while the 34,000 and 11,000 dalton proteins are unidentified but are not derived from serum.

King et al. (1975) studied the time of appearance of apoprotein in amniotic fluid by an agglutination immunoassay. They found that the apoprotein was first detected at 30 to 32 weeks of gestation, and its concentration increased almost fivefold to a maximum at 37 weeks. At all gestational ages there was wide variability in both phospholipid and

apoprotein concentrations, and the time of appearance of the apoprotein in amniotic fluid differed among fetuses. They also found that the presence of surfactant apoprotein in amniotic fluid is coincident with the biochemical and morphological maturation of the fetal lung and this protein is cosecreted with the lipids of surface active materials.

Pulmonary surfactant and lung maturation

The mammalian lung undergoes changes late in gestation that are essential for the newborn infant to survive. Brumley et al. (1967) studied the development of lung at 120 to 130 days in lamb and found that phospholipid and percent saturated fatty acids on phosphatidyl choline were relatively constant from 60 to 120 days gestational age; thereafter there was a significant increase in both measurements. The trigger for lung maturation is a result of the effect of steroids upon the fetal lung. Liggins (1969) observed that hydrocortisone infusion into the fetal lamb led to premature delivery and these prematurely born lambs were viable at an earlier gestational age than lambs that had not received the steroids.

Kontas and Avery (1971) injected a single dose of 9-fluoroprednisolone intramuscularly and intraamniotically at 24 days of gestation in a rabbit. At 26 or 27 days, in injected fetuses, the lungs were more mature than expected at that age, as reflected in the surface tension of lung extracts and pressure-volume curves. Wang et al. (1971) histologically studied the rabbit lung by injection of 9-fluoroprednisolone into 24 day rabbit fetuses. They found that the steroid induced accelerated maturation of the alveolar lining cells and at 26 or 27 days gestation, there was in-creased formation of osmiophillic bodies in type II alveolar cells and abundant osmiophillic material in the alveolar spaces. The alveolar cells

at this time were similar in appearance to those of 28 and 29 day control fetuses. There are receptors for steroids present in the fetal lung in greater quantity per unit mass than in any other organ. The receptors bind the steroid and probably transport it to active sites within the cells (Ballard and Ballard, 1972). Adams and Fujiwara (1963) reported that the immature lamb lacked surface activity in the tracheal fluid, whereas the mature fetus had detectable surfactant in the tracheal fluid. It appeared that the surfactant enters the amniotic fluid of the mature fetus. Scarpelli (1968b) was the first who indicated the importance of amniotic fluid studies to estimate the lung maturity. Gluck et al. (1971) developed the method to predict the respiratory distress syndrome in the newborn. The infant mortality and the respiratory distress syndrome are closely related to lung immaturity and probably to inadequate surfactant secretion. Gluck observed that during maturation the lung has little change in sphingomyelin content but a great increase in saturated phosphatidyl choline. By this observation it can be assumed that the lecithin and sphingomyelin in the amniotic fluid would be determined largely by the secretion of surfactant from the lung. Gluck et al. (1971) established the ratio between lecithin/sphingomyelin in the amniotic fluid for prediction of the respiratory distress syndrome of the newborn. They concluded that if a ratio of 1.5 or lower is present in amniotic fluid within 24 hours of birth, a high incidence of respiratory distress syndrome occurs. In contrast, if the lecithin/sphingomyelin ratio is above 2.0 a low incidence of respiratory distress syndrome occurs. The measurement on amniotic fluid can therefore be used to predict the respiratory distress syndrome if the fetus is delivered within the next 24 hours.

This method is supported by the report of Blumenfeld et al. (1974).
These observations also suggested that lung immaturity with inadequate
secretion of saturated phosphatidyl choline causes respiratory distress
syndrome.

Glycoprotein

A great number of the proteins that are found in nature have carbo-
hydrate covalently linked to the peptide portion and are termed glycopro-
teins. These conjugate proteins are represented by many substances of
biologic importance, including enzymes, hormones, antibodies and mem-
branes. The collagens, major structural proteins of the body, have now
been clearly shown to belong to the glycoprotein family, and a large
number of carbohydrate-containing proteins are present in plasma and
mucous secretion. Glycoproteins have no unique amino acid composition,
but do contain carbohydrates such as D-mannose, D-glucose, L-fucose, D-
xylose, N-acetyl-D-glucosamine and the various derivatives of neuramimic
acid (the sialic acids). The carbohydrate content of glycoproteins may
vary from less than 1 percent to more than 80 percent of the weight of the
molecule, and as few as two to as many as seven sugar types may be present
in a given glycoprotein (Spiro, 1963).

Many of the plasma proteins that play a part in transport have been
shown to be glycoproteins, including ceruloplasmin, transferrin, hapto-
globin, thyroxine-binding protein and corticosteroid transporting glyco-
protein (Seal and Doe, 1962). Chandrasekhar et al. (1962) studied the
glycoprotein in the prothrombin and fibrinogen. They suggested that the
sialic acid component of fibrinogen may have a role in regulating the

clotting mechanism. Kabat (1956) reported that the blood group substances are glycoproteins with antigenic properties. It has become apparent that the serologic specificity of the blood group substances resides in their carbohydrate portion which consists of approximately 75 percent of the molecule and the sugars present are fucose, galactose, glucosamine and galactosamine. The differences in amino acid composition do not appear to influence the immunologic specificity whereas alteration in carbohydrate structure has a marked effect on antigenic properties. Clamp and Jones (1968) have shown that the oligosaccharide units of a soluble glycoprotein have antigenicity as assessed by inhibition of passive hemagglutination and passive cutaneous anaphylaxis reactions. The glycoproteins have been found in the mucous secretion of the salivary glands, the bronchi, the stomach, the cervix and the bile ducts. The viscous properties of this secretion have been attributed to the physical properties of the glycoproteins present. These glycoproteins from epithelial mucous secretions contain as their sugar constituents sialic acid, fucose, galactose, glucosamine and galactosamine. Gottschalk (1969) reported the high viscosity of the ovine submaxillary gland glycoprotein has been attributed to the presence of negative charges of the sialic acid residues in the disaccharide units distributed along the length of the peptide chain. With selective removal of the sialic acid residues with neuraminidase, there is a marked decrease in the viscosity.

Serum glycoprotein

The level of glycoproteins in serum is relatively constant in an individual under normal conditions, but is changed slightly by even minor ailments. Certain pathological conditions such as cancer, rheumatoid

arthritis, tuberculosis are characterized by elevated serum glycoprotein and seromucoid levels (Winzler, 1955; Shetlar, 1961). Weimer and Humelbough (1965) have found that food restriction and protein depletion in rats brought about a decrease in protein-bound hexose (PBH) and seromucoid. Davis and Richmond (1968) reported that hexosamine and sialic acid components of glycoprotein in serum were reduced in induced protein deficiency in the rat. Patwardham et al. (1971) reported that the protein-bound hexose (PBH), α-glycoprotein and seromucoid were markedly elevated in the protein-calorie deficiency disease in children. Bogden et al. (1967) found that the α-2 glycoprotein level increased in adjuvant-induced polyarthritis. Bogden and colleagues (1968) studied the relation between glycoprotein synthesis and concentration of steroids, and found that the synthesis of a specific glycoprotein is a quantitative reflection of trauma. They observed a linear increase in serum glycoprotein concentrations in traumatized adrenalectomized animals receiving increasing dose levels of cortisol. They suggested that glycoprotein synthesis can be used as a sensitive and specific assay for the study of anti-inflammatory activity of both natural and synthetic 11-oxygenated corticosteroids as shown by the parallelism between dose-response curves obtained and the relative potencies of these compounds as anti-inflammatory agents. Tobiska et al. (1969) also reported that the abscess induced by injecting a sterile turpentine oil into germ-free rats elevated the concentration of glycoproteins and seromucoids.

Patterson et al. (1963) found that the carcinosarcoma induced the elevation of the total serum-bound carbohydrate to increase by the third day prior to visible growth of the tumor. The serum-bound carbohydrate

remained elevated throughout the experiment. Snyder and Ashwell (1971) also reported the serum concentrations of glycoprotein and serumucoid were significantly increased during malignancy in man. Murphy et al. (1972) showed a marked increase of glycoprotein in malaria and they proposed that the serum glycoprotein change was the result of an initial inflammatory response. Mousa et al. (1973) also reported the increased serum glycoprotein levels in acute and chronic malaria in man. Mouser et al. (1976) found that the glycoprotein electrophoretic pattern was significantly altered in schistosomiasis; α-1 and γ-glycoprotein were significantly elevated above the normal concentrations. Dugan et al. (1968) found that the influence of estradiol on immature rat uteri caused a significant increase in total protein and sialic acid.

Glycoproteins of cell surface

The presence of glycoprotein on the surface of mammalian cells is well-documented (Cook, 1968). Rombourg and Leblond (1967) used periodic acid-silver methenamine, a technique specific for glycoprotein detection. They found that nearly all cells were coated by a thin layer of stained material and this structure contained not only glycoproteins but also acidic residues. They pointed out that carbohydrates may play a role in holding cells together and in controlling the interactions between cells and environments. The carbohydrates possess unique immunological, cementing, and other properties, which may vary from cell type to cell type. Glossmann and Neville (1971) studied the glycoprotein of cell surface membrane from the liver, kidney, and brush border of the rat and found

that there are four subunits which have molecular weights from 96,000 to 250,000 all are prominent Schiff-positive glycoprotein containing sialic acid.

A role of glycoprotein in specific cell adhesion is also suggested by Margoliash et al. (1965) who found a high uptake and concentration of glycoprotein on the cell surface. Oppenheimer et al. (1969) showed that the intercellular adhesion depended on the incorporation of sugar into macromolecules on the cell surface. They assumed that the process of cell recognition and cell adhesion required glycoproteins. Springer and Ansell (1958) investigated the effect of influenza viruses and receptor-destroying enzyme from Vibrio cholerae upon serologically recognized receptors of human erythrocytes. They found that influenza virus and receptor destroying enzyme are capable of inactivating or removing blood group agglutinogens M and N from human erythrocytes. Ambrose and Roe (1966) have suggested that the increased sialic acid content of malignant cells, compared to normal, may be responsible for the loss in affinity of these cells for each other and, therefore, for the metastasis of various cells. However, the plasma membrane glycoproteins have been implicated as participating in the mutual recognition and adhesiveness of like cells to form aggregates of tissue.

Rosen et al. (1958) first described the disease called pulmonary alveolar proteinosis which is characterized by excessive accumulation of periodic acid-Schiff (PAS)-positive amorphous material within the alveoli. Kuhn et al. (1966) observed the proliferation of the alveolar type II cells in lungs of patients with alveolar proteinosis and suggested that

the intra-alveolar material was derived from degenerated or desquamated alveoli cells. Lambson and Cohn (1968) studied the ultrastructure of the lung of the goose and its lining of surface material. They found that avian atria and respiratory bronchioles are lined by a multilaminated membrane surface. Osmiophilic inclusions are found in the type II epithelial cells of the atria, but unlike mammalian lungs, no Clara, globlet or ciliated cells are present in avian airways. Bhattacharyya et al. (1975) isolated glycoproteins from lung lavage of rabbits and found the glycoprotein had molecular weight of 62,000 and 36,000. They pointed out that these two collagen-like glycopeptides are major intra-alveolar proteins in many mammals including man. Bhattacharyya et al. (1976) studied the glycoprotein from lavage of normal chicken lungs and found it to have molecular weight of 36,000 daltons. They suggested that this glycoprotein may be a product of type II cells, since the chicken lung contained only alveolar type II cells.

N-Acetyl Neuraminic Acid (Sialic Acid)

The sialic acid occurs as a constituent of mucoproteins, mucolipids and lipoprotein-carbohydrate complexes in animal tissues, and is found in glandular secretion, excretions, blood serum and as a constituent of cell surface (Gottschalk, 1960). The sialic acids have received special attention as normal constituents of various glycoproteins. Among them, certain circulating sialoglycoproteins called "acute-phase reactant" proteins increase in concentration in the serum in a number of pathologic states.

The pathologic changes can be detected by the increased level of sialic

acids in sera of patients.
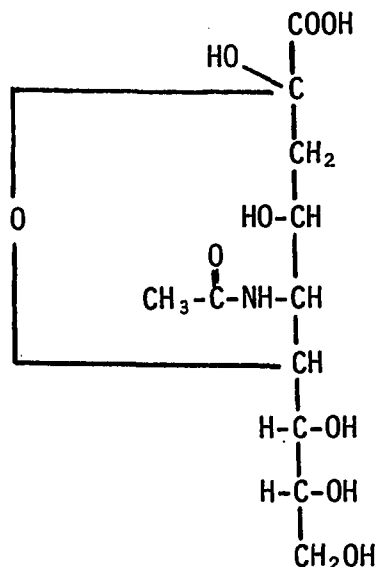
Mol. Wt. 309.3 ($C_{11}H_{19}O_9N$)

N-acetyl neuraminic acid

There are many methods available for determining sialic acids in biologi-

cal materials. The direct histochemical method for the demonstration of

sialic acid was developed by Shear and Pearse (1963). Svennerholm (1958)

developed the resorcinol procedure for the determination of sialic acid in

body fluids such as serum, lymph, milk, synovial fluids and transudates.

Warren (1959) developed a very sensitive method for determination of

sialic acid. This method involves the use of thiobarbituric acid as a

reagent and is relatively specific for free sialic acids. Engen et al.

(1974) adapted this method to the autoanalyzer for measuring sialic acid

and 2-deoxy ribose in blood and tissue samples. Gottschalk (1960) ob-

served that human serum contains no free sialic acid and that 90% of the

serum sialic acid is bound to the α and β-globulin, and the only member of

the sialic family so far identified in human serum is the N-acetyl derivative of neuraminic acid (NANA). Carter and Martin (1962) reported the increase of sialic acid level in rheumatoid arthritis, cirrhosis, myeloma and macroglobulinaemia. The increase in serum sialic acid levels is associated with the presence of excess of 19S globulin. Since 90% of the sialic acid in normal sera is bound to the α and β-globulins it is reasonable to anticipate that under conditions in which these fractions of the serum protein pattern are increased, there may also be a raised level of sialic acid. Singh and Ramraju (1967) used Warren method to survey the sialic acid level in serum of healthy Indian adults and found the average value was 56 mg % with no difference by age or sex. Singh et al. (1967) reportd the significant increase of serum sialic acid in the malignancies but McClelland and Bridges (1973) found a lower sialic acid content in lymphocytes from patients with chronic lymphatic leukemia than in the normal lymphocytes. Serum sialic acid values also increase in cancer, leukemia, and pneumonia (Gottschalk, 1960).

Fuller and Engen (1971) reported the sialic acid levels in normal beagles ranged from 55.8 to 59.3 mg %. Engen (1971) reported the serum sialic acid is significantly increased in the dogs infected with canine distemper. The serum sialic acid concentration in normal dogs was 50.9 mg % as compared to 79.7 mg % in distemper infected dogs.

Some biological activity of sialic acid

Several factors indicated that analysis of the sialic acid composition represented an ideal way in which to begin a broad survey of the comparative biochemistry in some hormones. Sialic acid is relatively

easily removed from the protein by treatment with enzyme neuraminidase (Gottschalk, 1960). There are many workers who have shown that sialic acid is an important part of the carbohydrate moiety with respect to the biological activity of the molecule of mammalian gonadotropins. Licht and Papkoff (1972) found that by desialylation of gonadotropins with neuraminidase the loss of activity was between 70 and 90% and all mammalian and avian gonadotropins were inactivated. The desialylation of ovine or human FSH by neuraminidase destroys most of the activity of the hormone in vivo as indicated by mammalian bioassay (Papkoff, 1965; Amir et al., 1966). In contrast, luteinizing hormone (LH, ICSH) of ovine, bovine, and porcine origin lack sialic acid and are unaffected by neuraminidase (Adams-Mayne and Ward, 1964; Papkoff, 1966). Coppola and Ball (1966) reported the uterine sialic acid concentration in ovariectomized rats was directly proportional to the level of estrogen administered and inversely proportional to that of progesterone. Rajalakshmi et al. (1969) showed that estrogen induced a sharp increase in uterine sialic acid and the level of sialic acid 6 hours after clomiphene (estrogen antagonist) treatment was significantly less than that caused by estrogen at the same time period. However, Kennedy and Emmens (1975) reported that progesterone was found to increase uterine sialic acid concentration above that induced by estradiol-17β alone in ovariectomized rats.

Sinha and Melnykovych (1972) found that sialic acid is released into the culture medium in the form of sialopeptides and that the accumulation of these sialopeptides in the medium is decreased when cells are grown in the presence of prednisolone. The synthesis of sialopeptidase was

depressed by prednisolone (Sinha and Melnykovych, 1973). They also pointed out that the surface glycopeptides are involved in the control of growth in cultures of epithelioid cells of neoplastic origin. Kraemer (1966) and Carubelli and Griffin (1967) found that the sialic acid content of Hela cells growing exponentially in monolayer cultures increased with the presence of hydrocortisone. Engen (1970) reported the serum sialic acid level is significantly increased in the dog treated with hydrocortisone and dexamethasone.

Hirst (1941) was the first who found that a virus can adsorb to chicken red blood cells. The influenza virus and Vibrio cholerae possess enzyme neuraminidase. The action of neuraminidase renders the red blood cells unable to adsorb virus by removal of the specific receptors (Gottschalk and Lind, 1949). The product removed is sialic acid of which N-acetyl neuraminic acid is the principal form in virus-binding mucoprotein (Zilliken et al., 1957). Gottschalk (1956) showed that the sialic acid moiety of mucoprotein occupies a terminal position in an oligosaccharide. When mammalian cells are infected with certain viruses, especially myxo- or paramyxo-viruses, the virus particles are adsorbed to receptors such as the sialic acid residues (Haff and Stewart, 1965).

Levenson et al. (1969) showed that the sialic acid was the active substituent on the horse serum inhibitor of influenza virus, and profound changes in the inhibition of the virus occurred when a chemical agent such as sodium metaperiodate was used, which makes relatively minor alterations of the sialic acid. Gottschalk (1966) discussed the criteria that glycoproteins must fulfill to be effective inhibitors of influenza

virus hemagglutination, and he concluded that the effectiveness of an inhibitory substance is a function of molecular size, the number of terminal sialic acids and the three-dimensional structure complementary to an area on the virus surface.

Van Herick and Eaton (1945) reported the hemagglutination of turkey and blood cells by Mycoplasma gallisepticum. Gesner and Thomas (1965) demonstrated that the hemagglutination of turkey erythrocytes by Mycoplasma gallisepticum was inhibited by mucoproteins containing sialic acid, or by sialic acid itself, or by treatment of the erythrocytes with neuraminidase. They suggested that sialic acid on the erythrocyte surface provides binding sites for Mycoplasma gallisepticum. Mycoplasma pneumoniae is the most virulent of the mycoplasmas for humans (Chanock, 1965). It has been suggested that its virulence is due, in part, to a peroxide hemolysin secreted by this organism which diffuses to the cell membrane and thus enhances injury of affected cells (Cohen and Somerson, 1967). Sobeslavsky et al. (1968) showed clearly that monkey, rat, and chicken tracheal epithelial cells, as well as monkey, rat, guinea pig and chicken erythrocytes adsorbed firmly to colonies of Mycoplasma pneumoniae and Mycoplasma gallisepticum. Mycoplasmas appeared to bind to neuraminic acid receptors on erythrocytes or tracheal epithelial cells as pretreatment of red cells or tracheal epithelial cells with either receptor-destroying enzyme neuraminidase or influenza B-virus, removed the adsorption receptor for M. pneumoniae. Pretreatment of M. pneumoniae colonies with neuraminic acid containing materials prevented adsorption of erythrocytes or respiratory tract cells. Sobeslavsky and collaborators pointed out that the

affinity of M. pneumoniae for respiratory tract epithelium may play a role in virulence since this type of attachment provides an unusual opportunity for peroxide, secreted by M. pneumoniae to attack the tissue cell membrane without being rapidly destroyed by catalase or peroxidase present in extracellular body fluids.

Pulmonary Function Tests

Wilson et al. (1976) suggested that the methods for measuring of the pulmonary functions can be divided into the following categories: 1) ventilatory exchange, a measurement of volumes of gases exchanged during breathing; 2) static lung volumes, providing useful information about the strength and elasticity of the respiratory system; 3) respiratory mechanics; 4) distribution of ventilation; 5) pulmonary circulation including the measurement of vascular pressures; 6) regional ventilation/perfusion matching $(\dot{V}/\dot{Q})$; 7) diffusion capacity which is affected by a variety of lung diseases; and 8) blood gas measurement. Few respiratory parameters can be measured in animals because of their lack of cooperation. MacFarland (1976) suggested the subjects to be measured in animals as the following: 1) pulmonary mechanics, including respiratory rate, tidal volume, minute volume, airway resistance, and pulmonary compliance; 2) distribution of ventilation; 3) diffusion capacity; and 4) blood gases and pH.

The review on pulmonary function tests is subdivided into five components: 1) lung volume; 2) ventilation; 3) pulmonary mechanics; 4)

distribution of ventilation; and 5) pulmonary circulation and blood gas analyses.

Lung Volume

For the past century the total lung capacity (TLC) and its subunits have been measured by a spirometer. The spirometer has also been used for the routine measurement of basal metabolic rate (BMR). Bates et al. (1971) noted that in order to measure absolute gas volume in the lung, one of three different methods must be employed: inert gas dilution or wash-out, whole body plethysmography, or radiological techniques.

Tidal volume

Tidal volume (V_T) is the volume of gas inspired or expired during a single respiratory cycle. Comroe et al. (1962) suggested that tidal volume is not useful as an index of alveolar ventilation because either increased or decreased tidal volume may be associated with hyper- or hypoventilation, depending on other factors. However, when tidal volume is considered along with frequency, it is possible to diagnose hypoventilation without the necessity of other tests. Tidal volume is primarily of importance because it is one of the determinants of "minute volume," the volume of gas pumped into and out of the upper airways per minute (tidal volume x respiratory frequency = minute volume).

Alveolar Ventilation

The alveolar ventilation can be calculated by subtracting the dead space ventilation from the minute ventilation.

Alveolar ventilation = Respiratory frequency x (tidal volume - dead
space volume)

In man, when $\dot{V}/\dot{Q}$ ratios are similar throughout the lungs, as in

normal subjects, an alveolar ventilation at rest of about 4 liters per

minute is adequate. When the $\dot{V}/\dot{Q}$ ratios are unbalanced throughout the

lung or when the lung is destroyed by disease, the alveolar ventilation

will be greater in order to maintain normal arterial gas tensions. The

only satisfactory method for the measurement of effective alveolar venti-

lation is by the measurement of arterial CO_2 tension (Bates et al., 1971).

Forster (1974) suggested that the minute volume which included the dead

space and the effective alveolar ventilation should not be considered as

an effective indicator of ventilation efficiency for respiratory gas ex-

change, because there is no significant gas exchange with blood occurring

at "anatomical respiratory dead space" which includes pharynx, larynx,

trachea, bronchi and bronchioles. Respiratory dead space or physiological

dead space (V_D) is the value of lung which has no gas exchange and is made

up of two compartments: anatomic dead space and alveolar dead space which

is the nonfunctioning alveoli. V_D can be determined by the Bohr's equa-

tion as described by Ruppel (1975) or a single breath analysis method as

described by Buist (1975). However, the size of anatomic dead space

varied with the size and posture of the individual (West, 1974). In

mammals the size of the anatomical dead space of a normal individual in

milliliters is approximately numerically equal to its weight in pounds

(Forster, 1974; Ganong, 1975).

A decrease in alveolar ventilation causes CO_2 retention. A lower

ventilation occurs in several diseases such as muscular weakness,

poliomyelitis, central depression from pharmacologic agents as in general anesthesia or intoxication or increase in physiologic dead space in emphysema (Forster, 1974). The magnitude of arterial PCO_2 depends on pulmonary function, including the frequency of respiration. Thus, an increased arterial PCO_2 indicates a decrease in alveolar ventilation (hypoventilation) and decreased arterial PCO_2 indicates an increase in alveolar ventilation (hyperventilation). With modern equipment for blood gas analysis the combination of blood gas analysis and the measurements of ventilation become the best methods in clinical practice for evaluation of pulmonary function in man and animals as reported by Pickrell et al. (1971), Forster (1974), and Gillespie and Hyatt (1974).

Lung Mechanics

Lung mechanics involves the action of the lungs and thorax in pumping gas into and out of the alveoli. In general, the lung mechanics can be subdivided into four components: 1) dynamic lung compliance, 2) airway resistance, 3) collapse of the airways, and 4) work of breathing.

Lung compliance

Measurements of lung compliance provide only limited information as to the absolute elastic properties of lungs. Compliance is influenced by lung volume and respiratory frequency. Forster (1974) suggested that compliance is a very useful concept clinically because it gives a measure of the changes in lung elasticity with disease. A decrease in compliance of the lung means that the lung has become stiffer, as in fibrosis, pulmonary edema, or pleural thickening. Compliance can become greater when the lung

becomes more distensible, as it does in emphysema, presumably from loss of elastic tissue. The only adequate measure of the lung elastic properties is the dynamic pressure-volume curve of the lung, in which absolute transpulmonary pressure is related to absolute lung volume over the whole vital capacity range, on both inflation and deflation. If volume is measured plethysmographically or by integration of the pneumotachograph signal and the transpulmonary pressure measured by insertion of esophageal balloon, dynamic compliance can be determined (Fry et al., 1952). Changes in intra-esophageal pressure are equal to changes in intrapleural pressure (Milic-Emili et al., 1964; Dubin, 1970; Gillespie and Hyatt, 1974). The dynamic lung compliance equals the slope of a graph of lung volume vs. intrapleural pressure. In patients with emphysema the curve is shifted upward and to the left indicating a marked loss of elastic recoil. In asthmatic subjects with bronchospasm there is also loss of recoil (Gold et al., 1967; Woolcock and Mead, 1968). In patients with pulmonary fibrosis the curve is shifted downward and to the right, indicating a marked increase in elastic recoil at all lung volumes. There are many factors contributing to the shape and position of the static pressure volume curve of the lung (Figure 1). There is a surface tension at the tissue-gas boundary which tends to collapse the alveoli, and which increases the force required to expand the lung (decrease the compliance). Pulmonary surfactant, dipalmitoyl lecithin complexed with protein, has been extracted from saline lavage of normal lung and is essential to the stability of the lung and thereby effective ventilation and gas exchange

I **Normal**

II **Rapid Shallow Breathing**

III **Increased Airways Resistance**

IV **Decreased Compliance with Atelectasis**

Lung Volume (vol)

pressure (p)

slope $\dfrac{\delta V}{\delta P}$ =compliance

area of loop= work performed

expiration

inspiration

Vol.

P

rate increases tidal volume falls

Vol.

P

fat loops indicates increased work

Vol.

P

$\dfrac{\delta V}{\delta P}$ is reduced

and work is increased
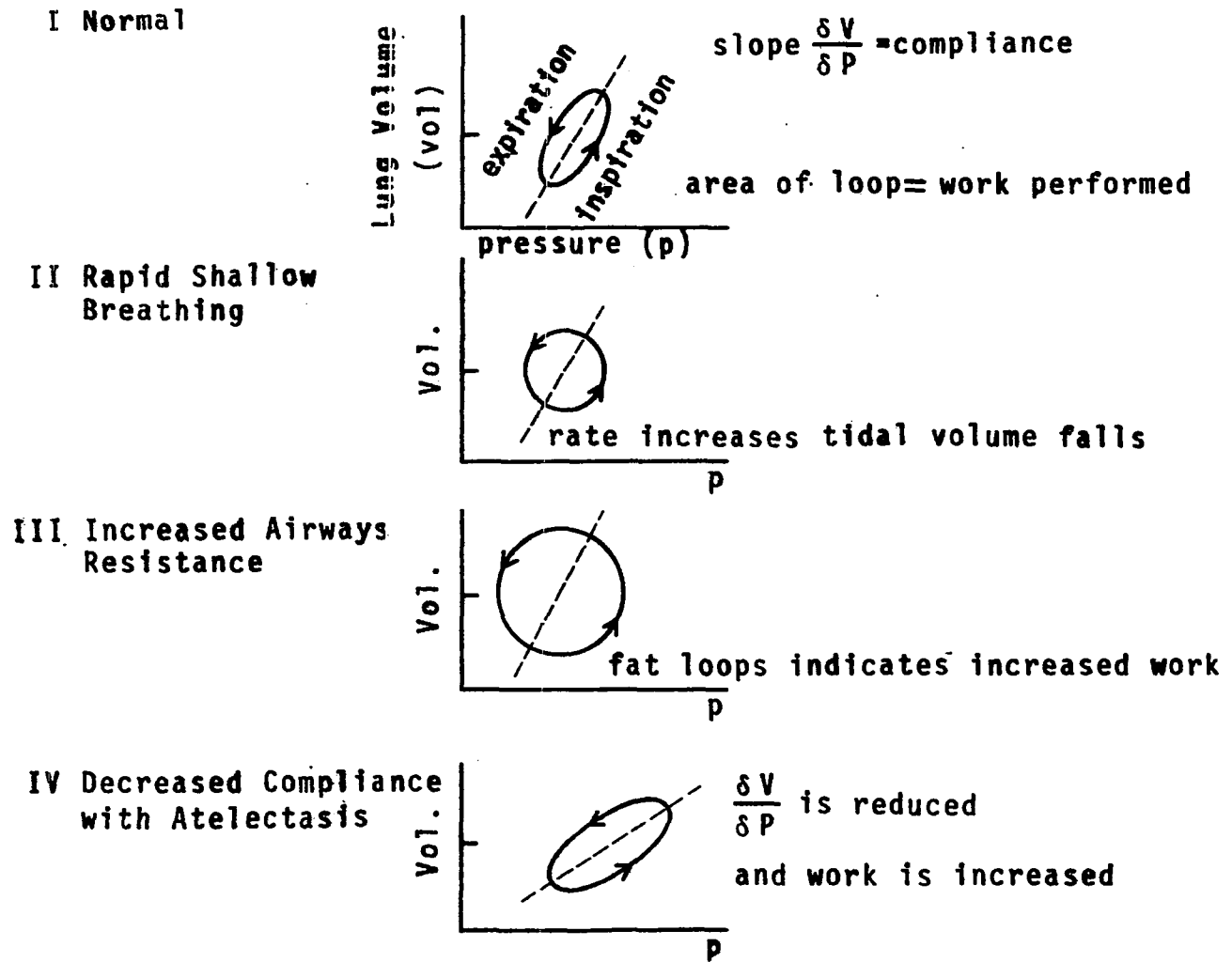
Figure 1. Pressure: volume loop diagrams relating change of lung volume of transpulmonary pressure (P).

(Dubin et al., 1971). In premature infants the pulmonary surfactant is decreased as compared to full term infants (Gluck et al., 1971).

Airway resistance

Airway resistance is the most exact measurement of airway obstruction. The calculation of airway resistance requires simultaneous measurement of air flow and alveolar pressure as estimated by esophageal pressure. Airway resistance and tissue resistance contribute to total pulmonary resistance, but airway resistance contributes most of the total resistance (Forster, 1974). Airway resistance has been shown to be dependent upon two factors:

1. Lung volume: The airway resistance varies inversely with lung volume. The change with lung volume is due to the increase in diameter of the airways as the lung volume increases. The elastic elements in the lung are attached not only to the pleural surface but also to the outer walls of airways and extra-alveolar blood vessels with the result that peribronchial pressure is equal to or more negative than pleural pressure (Hyatt and Flath, 1966). In dogs, the greatest changes in resistance with lung volume occur in 3 to 8 mm diameter airways (Macklem et al., 1969).

2. Relative resistance of central and peripheral airways: The contribution of different sized airways to the total pulmonary resistance has been measured in dogs (Macklem and Mead, 1967) and in man (Hogg et al., 1968).

According to the Poiseuille relationship for viscous flow, airway resistance should decrease as the fourth power of the effective radius of the airways (Comroe, 1974). The resistance of the upper airway is about 50 percent and that of the peripheral airways about 5 to 15 percent of total pulmonary resistance (Hyatt and Wilcox, 1961; Ferris et al., 1964). Hogg et al. (1972) demonstrated little increase in total airway resistance in chronic obstructive airway disease patients but a fourfold increase in peripheral resistance.

Collapse of the airways

McCarthy et al. (1972) and Forster (1974) reported that even in healthy individuals, some bronchioles close when lung volume is reduced during expiration to 5 to 10 percent of vital capacity. This is called "closing volume." Holland et al. (1968) reported the airway closure occurred at the level of the resting Functional Residual Capacity (FRC) in man ages 66 and 75. Anthonisen et al. (1969) reported that the closing volume at age 20 is at a lung volume less than 10 percent of the vital capacity, but at age 40 closing occurs at about 20 percent of vital capacity.

The work of breathing

Fenn (1951) first drew attention to the various factors involved in the work of breathing. Subsequently the work of breathing has been extensively reviewed by Otis (1954). Energy is required to pump the minute ventilation into and out of the lungs. The work is done by the diaphragm and intercostal and accessory muscles of respiration. Normally these muscles do most or all of their work during inspiration, overcoming the

elastic force of the lung and thoracic cage as well as the viscous re-sistance of gas flow through the bronchial tree. During expiration the elastic recoil of the lung and thoracic cage exerts a force tending to return the lungs to resting and expiratory level (FRC), so that no muscular effort is normally needed. During a forced expiration or in disease in which expiratory resistance is increased, muscular effort is required. In general the work of breathing is about 5 percent of the resting metabolic rate (Forster, 1974).

During steady-state breathing conditions it is possible to compute the work of breathing in mechanical terms as the relationship of pressure (which is an expression of force) to volume (which is an expression of distance). Since pressure x volume (g/sq cm x cucm = g x cm) has the same dimensions as work (force x distance), it is most convenient to measure work of breathing as pressure x volume (Ganong, 1975). Mammals of very different size naturally breathe at rates and depths that represent their own minimal rates of respiratory work as demonstrated by Crosfill and Widdicombe (1961). Widdicombe and Nadel (1963) explored the interrela-tionship that exists between anatomical dead-space volume and work of breathing at given levels of alveolar ventilation and suggested that alterations in airway caliber might represent adjustments to ensure maintenance of a minimal work of breathing.

Distribution of Ventilation

A common cause of hypoxemia in the diseased lung is the unequal distribution of ventilation to perfusion. Such inequalities primarily

arise from unequal distribution of ventilation to various lung regions.
There are a number of possible causes of unequal gas distribution in the
lung: 1) regional ventilation distribution; 2) relation between airway
resistance and ventilation distribution; 3) airway closure; and 4)
gaseous diffusion (Bates et al., 1971).

Regional ventilation distribution

Milic-Emili et al. (1966) studied the regional distribution of in-
spired gas in the lung in man by using [133]Xenon and found that regional
volumes were always greater in upper than in lower lung zones. They
pointed out that the uneven distribution of ventilation in the normal lung
is the consequence of regional differences in pleural pressure. Zardini
and West (1966) studied the topographical distribution of ventilation in
isolated dog lungs. When the lung was inflated in small steps, most of
the [133]Xenon initially went to the upper zone, but at larger lung volumes
the lower zone was preferentially filled. They concluded that the uneven
distribution of ventilation in the human lung is not caused by the uneven
distribution of blood flow but by the vertical gradient of the intra-
pleural pressure. The intrapleural pressures at different sites in the
thorax and the effects of different body positions on these pressures play
a role in pulmonary mechanics (Mead, 1961), and fluid dynamics (Agostoni
et al., 1957). Hydrostatic effects are of considerable importance in
cardiopulmonary circulation and may also affect intrapleural pressures.
Kaneko et al. (1966) reported that in all body position studies, the
regional RV, FRC, and ERV were greater in upper part of the lung that in
dependent lung regions. They pointed out that in normal men both

ventilation and perfusion are relatively greater in the direction of gravity. Bryan et al. (1966) studied the regional variations in lung volume and in distribution of ventilation by measuring ^{133}Xenon incorporation during normal gravity and during increased positive acceleration on a human centrifuge. They concluded that the probable cause of the regional differences in volume and ventilation which have been demonstrated is a gradient of static transpulmonary pressure down the lung. This gradient appears to be related to the weight of the lung. Bates et al. (1971) summarized that there is little doubt that regional ventilation distribution plays a major part in determining patterns of nonuniformity in lung ventilation under a wide variety of circumstances. The balance between ventilation and perfusion is achieved under resting conditions. Age, obesity, body position, acceleration, and weightlessness affect gas exchange.

Relationship between airway resistance and ventilation distribution

One of the most remarkable features of young normal lungs is that the millions of alveoli fill and empty synchronously during both slow and rapid breathing. Hogg et al. (1969) showed that in emphysema the resistance of collateral channels is very low, lower than airway resistance. In some patients with emphysema collateral channels are extremely important ventilatory pathways. Woolcock and Macklem (1971) reported that the collateral ventilation is rapid in the dog and man but is slow or absent in the pig. Macklem et al. (1969) concluded that the synchronous behavior is achieved in lungs in spite of time-constant inequalities of moderate degree because: 1) the resistance of peripheral airways is low; 2)

because of interdependence of lung units; 3) because in some species, ventilation of airspaces through collateral channels may occur just as rapidly as ventilation of these spaces through airways.

Airway closure

If breathing takes place at low lung volume, i.e., near residual volume, the upper lung units are better ventilated than those in the dependent zones. This reversal of ventilation distribution has been attributed to airway closure (trapping) in the dependent lung zone (Milic-Emili et al., 1966). The airway closure occurs when the pleural pressure must be greater than airway pressure (Dollfuss et al., 1967). As lung volume approaches residual volume (RV), the pleural pressure in the lower lung zones indeed exceeds airway pressure, causing trapping in the dependent lung zones. If then a small breath is inspired, the trapped units in the lower lung regions do not receive any of the inspired air. Consequently, the inspired air is delivered predominantly to the upper lung during slow and shallow breathing. Macklem and Mead (1967) found that the resistance of peripheral airways, i.e., airways smaller than 2 mm diameter, is only a small part of the regions. In normal young subjects airway closure in the dependent lung zone is present only at lung volume below the functional residual capacity (Milic-Emili et al., 1966). With increased age, however, the lung volume at which the airways close would be expected to increase because the lungs of elderly subjects lose elastic recoil (Turner et al., 1968). Hence, pleural pressure in the lower lung zones will exceed airway pressure at lung volumes higher in elderly subjects than in young subjects.

Gaseous diffusion

The actual exchange of gases in the lungs takes place by diffusion between the alveolar gas and capillary blood. Although the importance of the process of gas diffusion between the alveoli and pulmonary capillary blood has been known for 50 years, the aspect of pulmonary function test has been clarified only since 1945 (Bates et al., 1971). Carbon monoxide (CO) has approximately 250 times as great an affinity for hemoglobin as does oxygen. An alternative gas for measuring diffusion is O_2, but because the capillary PO_2 rises during transit to approach equilibrium with alveolar gas, the estimation of mean capillary PO_2 is extremely difficult and inaccurate (Forster, 1974).

Carbon monoxide has been used extensively in the study of pulmonary diffusion because it 1) diffuses passively across the alveolo-capillary membrane, 2) combines tenaciously with hemoglobin, and 3) exerts an extremely low partial pressure in pulmonary capillary blood so that the alveolar carbon monoxide tension may be considered to be almost equal to the alveolar-arterial carbon monoxide gradient. A number of carbon monoxide techniques have been proposed (Forster, 1957).

The basic diffusion equation for CO

$$DL_{co} = \frac{\dot{V}_{co}}{Pa_{co} - Pc_{co}}$$

where

DL_{co} is the diffusing capacity of the lung for CO, the unit was ml/min/mmHg.

Pa_{co} is alveolar CO tension.

Pc_{CO} is capillary CO tension, this value is usually extremely low and
may be disregarded.

$\dot{V}_{CO}$ is carbon monoxide uptake.

There are many factors affecting diffusion in a single alveolus: 1)
thickness of alveolar lining membrane; 2) thickness of alveolar lining
cell; 3) permeability of capillary wall; 4) thickness of layer of plasma
membrane between capillary wall and red blood cell; 5) permeability of
red cell membrane to CO or O_2; 6) reaction rate of hemoglobin with CO and
O_2; 7) presence of COHb in the pulmonary artery blood, which will diminish
rate of CO transfer. Ogilvie et al. (1957) suggested that the pulmonary
diffusion capacity can be used to detect the obstructive disorders of the
pulmonary circulation by using a rapid, single-breath screening test of
pulmonary diffusing capacity. For the test the patient inhales a low,
nontoxic concentration of carbon monoxide, holds the breath for 10
seconds, and then exhales. The single-breath D_{CO} method can be used for
measuring gaseous diffusion capacity in normal subjects and in heart dis-
ease (Bedell and Adams, 1962), acute pulmonary tuberculosis (Williams
et al., 1961), anemia (Rankin et al., 1961), asbestosis (Williams and
Hugh-Jones, 1960), and emphysema (Apthorp and Marshall, 1961).

Pulmonary Circulation/Blood Gas Analysis

The hemodynamics of the pulmonary circulation are complicated because
the vascular resistance is influenced by three extravascular pressures:
1) atmospheric pressure, since if the blood pressure within the capillar-
ies becomes less than alveolar pressure the vessels will collapse; 2)

intrapleural pressure, because as intrapleural pressure falls in relation to ambient pressure with inspiration, pressure in the right and left sides of the heart also falls; and 3) pressure in the abdominal cavity, which surrounds the inferior vena cava and when it increases an increased pressure gradient is produced from abdomen to thorax and venous return rises (Forster, 1974).

Rahn and Farhi (1964) recognized the matching of ventilation and perfusion in the lung, and established the ventilation-perfusion ratio $(\frac{\dot{V}A}{\dot{Q}})$. If the ventilation and blood flow are mismatched in various regions of the lung, impairment of both O_2 and CO_2 transfer results. Forster (1974) observed that in the normal healthy lung capillary blood flow tends to be lower in the apices and greater in the bases. On the other hand, minute ventilation tends to be higher in the apices and lower in the bases. One important cause of this is that the weight of the lung makes inflation of an alveolus in the basal region more difficult. The net result is a high alveolar ventilation/capillary blood flow in the apices, with a corresponding decrease in PCO_2 and rise in PO_2, and the reverse result is obtained for the bases. The local mechanism for control of airway resistance and capillary blood flow operate to reduce the nonuniformity. In many lung diseases this delicate adjustment is deranged, such as by destruction of some capillaries or local increases in airway resistance. This results in an increased arterial PCO_2 and a decreased arterial PO_2. The most helpful, in many patients, estimate of alveolar ventilation is the measurement of the arterial PCO_2. A decreased inspired PO_2 leads to a decreased arterial PO_2, which in turn causes hyperventilation because of

stimulation of the chemoreceptors (carotid or aortic bodies). This lowers arterial PCO_2 secondarily. A decreased alveolar ventilation, whether from decreased minute ventilation or increased dead space volume, will lead to an increased arterial PCO_2 and a decreased arterial PO_2. A reduced pulmonary diffusing capacity will tend to decrease arterial PO_2, but will leave arterial PCO_2 normal. Uneven alveolar ventilation/capillary blood flow will reduce arterial PO_2 but leave arterial PCO_2 normal. As the disease progresses, the unevenness of distribution will become more severe and the arterial PO_2 will drop further and eventually the arterial PCO_2 will rise. Forster (1974) suggested that the situation in an actual patient is often a combination of metabolic and respiratory disturbance. It is necessary to know the values of the three variables in the Henderson-Hasselbalch equation in order to be able to determine a patient's acid-base status, that is arterial plasma HCO_3-concentration, pH and PCO_2.

Ruppel (1975) noted that because CO_2 diffuses twenty times as readily across the alveolar-capillary membrane as does O_2, small changes in ventilatory pattern of distribution of gas will show little change in $PaCO_2$. Since diffusion defects rarely raise $PaCO_2$, high CO_2 tension strongly suggests that advanced pulmonary disease is present. The measurement of pH and blood gas tension is useful for diagnostic procedure in animals as recommended by Feigl and D'Alecy (1972) and Pickrell et al. (1973, 1974). Loew and Thews found an average PaO_2 of 95 mmHg in adults between the ages of 18 and 30, falling to an average of 85 mmHg by age 35, and to an average of 75 mmHg at age 60 (Bates et al., 1971). These data

showed that the PaO_2 decrease occurs almost linearly with advancing age. However, the arterial PCO_2 is not affected by age.

Factors Influencing Pulmonary Function

The ventilation, distribution, diffusion and perfusion components are essential to accomplish the gas exchange and gas transportation. There are many factors contributing to the alteration of these components of lung function.

Age

In human, there have been many studies on the effect of age on pulmonary parameters. There are many reports in humans that the residual and functional residual capacity increased with increased age, but the total lung capacity showed little change (Comroe et al., 1962). Mitten et al. (1965) reported that the chest wall compliance in man decreased significantly with age. Turner et al. (1968) reported that in human the static recoil pressures reduced throughout the age range of 20 to 60 years. They also found that an increase in functional residual capacity/ total lung capacity (FRC/TLC) ratio increased with age. The loss of lung elastic recoil in the elderly is due to the decrease of chest wall compliance. Leblanc et al. (1970) found that the closure volume increased linearly with age, and in seated subjects older than 65 years and in supine individuals older than 44 years there is significant impairment of ventilation distribution to the dependent lung zones, which necessarily causes impaired gas exchange within the lungs. Gelb and Zomel (1975) studied the influence of aging on lung mechanics in healthy nonsmokers and found an increased airway collapsibility during maximum flow with in-

creased age. The airway resistance in man increased in the old age
(Ganong, 1975). It has been known for some years that elastic content of
the human lung is increased in older subjects which would tend to make the
structure more rigid (Pierce and Hocott, 1960a,b). There are important
changes in the thoracic wall with age, particularly progressive calcifica-
tion of the chondral cartilage (Pierce and Ebert, 1958). Pierce and Ebert
(1959) noted that the entire pressure-volume curve is shifted to the left
with age. It is clear that there are three effects of age: 1) loss of
elastic recoil, so that lung volume is greater at the same pressure dif-
ferential in the older lung; 2) shift of the curve over most of its
course; and 3) increase in the volume of air in the lung at zero trans-
pulmonary pressure (Turner et al., 1968). Anthonisen et al. (1969) noted
that the distribution of ventilation in the older subject is bound to be
"unstable" and very much dependent on the actual tidal volume taken and
the increased residual volume of age is due to premature closure of air-
ways due to loss of recoil. The ventilation distribution becomes de-
pendent on the magnitude of the tidal volume as the lung looses recoil with
advancing age.

There are many reports on the effect of age on the respiratory
parameters in animals. Davidson et al. (1966) studied the effect of aging
on respiratory mechanics and gas exchange in rabbits, and found the
significant increase of functional residual capacity with age. The static
compliance and elastic resistance increased in the old rabbit, signifying
loss of elastic recoil and increased airway resistance. The changes are
mild producing no disturbance of gas exchange. Mauderly (1968) reported a

significant increase in lung compliance with the older dogs. He observed that young dogs have higher ventilation and larger resting lung volume, and consume more O_2 per kilogram body weight than do older dogs. A change of blood gas partial pressure can be observed in old dogs. The young dogs had lower arterial pH and increased arterial CO_2 tension with no significant change in O_2 tension. Robinson and Gillespie (1972) found the older dog showed a decreased lung compliance and the changes in volume ratios in older dogs are similar to those seen in aging people. Robinson and Gillespie (1973) studied the mechanical properties of the lungs of aging dogs and found that with increasing age, there is an increase in the ratio residual volume/total lung capacity (RV/TLC) and plethysmographic functional residual capacity/total lung capacity (FRC/TLC). They suggested that the increase of FRC/TLC appeared due to both a loss of lung elastic recoil in dogs over 1500 days of age and an increase in the resting volume of the thoracic cage. Knutsen (1976) reported that there are significant decreases of lung compliance, and significant increases of airway resistance, work of breathing, and esophageal pressure in the older dog as measured during the sedative period.

Weight

In humans, the expected values for respiratory parameters as calculated on the basis of height and age of patient has been established (Comroe, 1974; Ruppel, 1975). Crosfill and Widdicombe (1961) studied the physical characteristics of the chest and lung, and the work of breathing in different mammalian species and found the lung resistance tended to increase with the size of the animal of each species. Stahl (1967)

studied the correlation between the body weight and lung volumes, lung compliance, and air flow in different sizes and species of mammals and found a correlation coefficient of between 0.99 to 0.90. Both Mauderly (1968) and Dubin (1970) also reported a significant increase of lung compliance with the heavier dogs. Knutsen (1976) reported the significant increase of tidal volume, minute volume, inspiratory and expiratory velocity and esophageal pressure with the increase of body weight in sedated dogs.

Sex

In humans, Comroe et al. (1962) and Forster (1974) pointed out the difference of respiratory parameter between male and female. Hamlin and Smith (1967) studied the respiratory parameters in healthy dogs anesthetized with sodium pentobarbital and found a significantly greater minute volume in male dogs, and the significantly larger tidal volume per kg body weight in female dogs. The weight of male and female dogs was similar, but the female dogs were significantly younger. Knutsen (1976) reported that there is no influence of sex on the respiratory parameters in the sedated dogs.

The pulmonary function tests are clinically used for screening respiratory diseases in small animals. However, there still are limitations, because of lack of cooperation from animals. Robinson and Gillespie (1970) suggested that is is convenient to divide respiratory diseases into three functional types.

1. Restrictive disease, the characteristics of restrictive disease being a reduced compliance and FRC. The disease can be due to

diffuse fibrosis within the lung, focal areas of consolidation or atelectasis or lesions occupying space within the chest. The reduction of compliance increases the work of breathing. The animals with restrictive disease minimize the work to maintain minute ventilation by rapid shallow respiration.

2. Obstructive disease, the characteristic of which is increased FRC, with or without increased compliance, and expiratory dyspnea. Normally expiratory phase is passive and depends on the elastic recoil of the lung. The increase of compliance is usually associated with atrophic alveolar emphysema.

3. Diffusion abnormality, which is the impairment of diffusion capacity due to a thickening of the alveolo-capillary membrane or a reduction in the number of functional pulmonary capillaries.

There is a limitation for the measurement of the pulmonary function in the swine in the present experiments. Only some of the respiratory parameters such as tidal volume, esophageal pressure, respiratory flow rate, O_2 consumption rate, and blood gas analysis can be measured under the anesthetic condition.

MATERIALS AND METHODS

Experimental Animals

All pigs were of mixed breeding. They were obtained from the respiratory disease-free herd maintained at the Veterinary Medical Research Institute, Iowa State University. The original stock was established from surgically derived pigs and the herd has been maintained in quarantine for at least 20 years. The pigs were housed in isolation units during the experiment. All the pigs were either Yorkshires or Hampshires and were 5 1/2 to 6 weeks of age at the beginning of the experiments. The pigs were fed a complete 16% protein grower ration free of antibiotics. Water was provided ad libitum by automatic waterers.

Experimental Inoculations of Pigs

In all trials pigs were held in isolation units for at least 3 days prior to inoculation to allow them to become accustomed to feed and surroundings. Each pig was given two ml of a 10% suspension of homogenized pneumonic lung in Eagle's medium. The inoculum was administered by intratracheal instillation with the head of the pig held in an up-right position as the culture fluid was dripped into the trachea. To make sure that the needle was inserted intratracheally air was drawn back from trachea. The pigs were kept under observation for a varying number of weeks as determined by the particular experiment. The pigs were observed for general appearance, breathing pattern, presence of coughing and eating behavior.

Protocol for Experiment 1

The pigs were divided into three groups, to be examined at the fourth, fifth or sixth week after intratracheal inoculation. The body weights were recorded at the beginning and the end of the period.

Pulmonary Parameters Measurements

At the end of each period, the pigs were anesthetized by intravenous injection of sodium pentobarbital[1] (30 mg/kg body weight). Atropine sulfate[2] (0.11 mg/kg) was administered intravenously. The animals were placed immediately into the prone position, and the laryngeal area was sprayed with a local anesthetic Cetacaine[3] to prevent the irritation when inserting the endotracheal tube. The animals were then intubated with an appropriate endotracheal tube (8 mm-10 mm diameter).

The polygraph multichannel (Beckman type R Dynograph) was used to record the EKG lead II (channel 1), values for esophageal pressure (channel 2), respiratory rate, inspiratory and expiratory velocities (channel 3), tidal volume (channel 4).

The intrapleural pressure changes were estimated with an esophageal balloon catheter. The balloon, 4.0 cm long and 1.0 cm in diameter containing 3 ml of water, was attached to the end of a polyethylene catheter. The catheter was connected to a Statham P23Db pressure transducer and

---

[1]Med-tech, Inc., Elwood, Kansas.

[2]Burns-Biotic Laboratory, Oakland, California.

[3]Cetylite Industries, Inc., Long Island City, New York.

pressure changes were recorded on the dynograph. The balloon was lubricated and inserted into the esophagus to approximately the lower middle third of esophagus. If the balloon was placed too caudally, the heart beat would show on the recording. Attempts were made to place the balloon in the same relative position in each pig in order to get the best expression of esophageal pressure. The measurement of airflow (inspiratory and expiratory velocity) was achieved by using a Fleisch #0 pneumotachograph having a linear response to 27 L/min. A Statham PM5 differential pressure transducer was connected to the pneumotachograph and recordings were made on channel 3 of the Beckman type R dynograph.

The tidal volume was determined simultaneously by electronic integration of the flow signal, and was recorded on channel 4.

The Hewlett-Packard 7035B X-Y recorder simultaneously measured the pressure-volume loop, in order to calculate the dynamic lung compliance, pressure-flow loop, for calculation of airway resistance, flow-volume loop, and to determine the inspiratory and expiratory velocity.

The values of tidal volume and respiratory rate were obtained directly from the recordings and lung compliance was determined as the ratio of tidal volume to the difference in intraesophageal pressure at points of zero flow during inspiration and expiration. The airway resistance was computed by dividing intraesophageal pressure by the maximum expiratory flow at end inspiration and end expiration at the instants of no airflow during breathing.

The Measurement of O_2 Consumption

The oxygen consumption was measured immediately after the measurement of respiratory parameters. The endotracheal tube was connected to a 9 liter spirometer[1] filled with oxygen. After a 5 minute equilibration period, oxygen consumption was recorded for 5-6 minutes.

Hematologic Procedure

Blood sample collection

The groin area was shaved and swabbed with 70% alcohol, the femoral artery and vein were surgically exposed and the long dwell needles were inserted. The skin was partly sutured closed to hold the long dwell catheter. A heparinized 2 ml glass syringe was attached. The arterial and venous blood samples were collected during the pulmonary function measurement. The syringes were sealed with the metal cap to exclude air bubbles and placed in an ice bath pending blood gas tension analysis.

At the end of the respiratory parameters measurement, a 50 ml plastic syringe with the dead space filled with heparin solution was used to collect about 35 ml of blood. The blood was centrifuged at 3000 rpm for 10 minutes and plasma removed. The plasma was stored in the freezer at -30°C for further analysis.

---

[1]Godard N.V., Bilthoven, Holland.

Blood cell counts

Erythrocyte (RBC) and leukocyte (WBC) counts were determined. Differential leukocyte counts were made on smears stained with Wright's blood stain, and absolute numbers were calculated.

Packed cell volume

Packed cell volume (PCV) was determined by the micro-hematocrit method using capillary tubes. The tubes were sealed with critoseal and centrifuged at 15,000 rpm for 3 minutes. The percent of packed red blood cells was determined.

Hemoglobin

The hemoglobin (Hb) concentration was determined by the cyanomethoemoglobin method.[1]

Plasma protein studies

The total plasma protein was determined with a direct reading refractometer.[2]

Plasma protein and glycoprotein electrophoresis

Plasma protein electrophoresis was performed with a Model 1400 electrophoresis system[3] according to the method described by Davis (1964). A 7 percent acrylamide gel and a tris-glycine buffer with a pH of 9.5 was used. The electrophoresed gels were stained by aniline blue black dye for 1 hour and electrically destained. The gels were then scanned with a

---

[1]Hycel, Inc., Houston, Texas.

[2]AO Instrument Co., Buffalo, New York.

[3]Canalco Co., Rockville, Maryland.

Gilford recording densitometer[1] to calculate relative percentages of each protein fraction.

Plasma glycoprotein electrophoresis was performed similar to plasma protein electrophoresis, but the pH of tri-glycine buffer was 10.6. The staining for glycoproteins in acrylamide disc electrophoresis was with alcian blue as described by Wardi and Michos (1972).

Blood chemical analysis

The serum sodium (Na), potassium (K), chloride (Cl), glucose, BUN, cholesterol, and triglycerides were determined by a Technicon auto-analyzer.[2] The procedures were as described in automatic analyzer analytical methods manual.

Blood gas tension and pH determination

Heparinized samples of blood as described under the section on blood sample collection were analyzed for pH and partial pressure of O_2 (PO_2) and CO_2 (PCO_2). The analyses were accomplished with a IL 513 pH-blood gas analyzer[3], in addition the HCO_3, Base Excess, CO_2 content information was also included on the printout.

Sialic acid determination

Blood, alveolar surfactant and lung tissue were hydrolized by 5% of trichloracetic acid in physiological saline and incubated in 80°C water

---

[1]Gilford spectrophotometer Model 250, Gilford Instrument, Oberlin, Ohio.

[2]Technicon Co., Terrytown, New York.

[3]Instrumentation Lab., Inc., Lexington, Mass.

bath for 1 hour. The solutions were cooled and were then centrifuged at 400xg for 10 minutes. The concentration of sialic acid and 2-deoxyribose in the supernatant was determined by the autoanalyzer. The procedures are described by Engen et al. (1974).

The Removal of the Lung

As soon as the determinations of respiratory parameters were finished, the animal was totally heparinized. Then the endotracheal tube was connected to a Harvard respirator. The thoracic cavity was exposed by cutting along the sternum. After thoracotomy, the anterior and posterior vena cava were clamped. Physiological saline was perfused through the right atrium and the dorsal aorta was opened to drain blood. All blood was removed from the lungs by perfusion with approximately 1 liter of physiological saline. The whole lung was carefully isolated by clamping the trachea and pulmonary blood vessels.

Observation of Gross Lesions

The lungs were examined for gross lesions. The gross lesions including consolidation, atelectasis and edema were recorded and photographed.

The Lung Lavage

The lung lavage was performed by using a three-way valve connected between the trachea and a 500 ml syringe. Five gentle lavages, each with 200 ml of physiological saline were completed. Each volume of saline was

injected and withdrawn two times before being collected in an ice-cooled bottle. The total lavage fluid was 1 liter. Attempts were made to apply the same strength of the force and speed for flushing for all lavages.

Collection of Pulmonary Surfactant

The lavage fluid was centrifuged at 400xg for 15 minutes to remove the mucus and debris. Then the supernatant was centrifuged at 44,300xg for 1 hour.

The sediment and supernatant following the second centrifugation were separately collected into dializing tubing and then were dialized against deionized distilled water at 4°C in order to remove NaCl. Completion of dialysis was determined by using a Barnstead purity meter[1] to detect sodium ion. Both the sediment and supernatant were lyophilized to dryness in a freeze-dry apparatus.[2] The dried samples were weighed and kept in the freezer at -30°C for further analysis (Figure 2).

Lung Tissue Collection

As soon as the lung lavage was finished, the lung tissue samples were collected. Samples from each lobe of the lung were cut into small pieces by scissors and were pooled together. A small volume of distilled water was added, and the lung tissue was further disrupted by ultrasound.[3] The

---

[1]Barnstead, Still and Sterilizer Co., Boston, Mass.

[2]Lyophilizer, freeze dry-5 type, Lab Con Co., Kansas City, Missouri.

[3]Heat Systems Co., Melville, New York.

Lavage fluid (1 liter)

centrifuged at 400 x g, 15 minutes

Sediment containing mucus
and debris was discarded

Supernatant

centrifuged at
44,300 x g for
1 hour

Sediment

Dialized against
deionized distilled
water

Ion free sediment

Lyophilized

Dried sediment

Supernatant

Dialized against
deionized distilled
water

Ion free supernatant
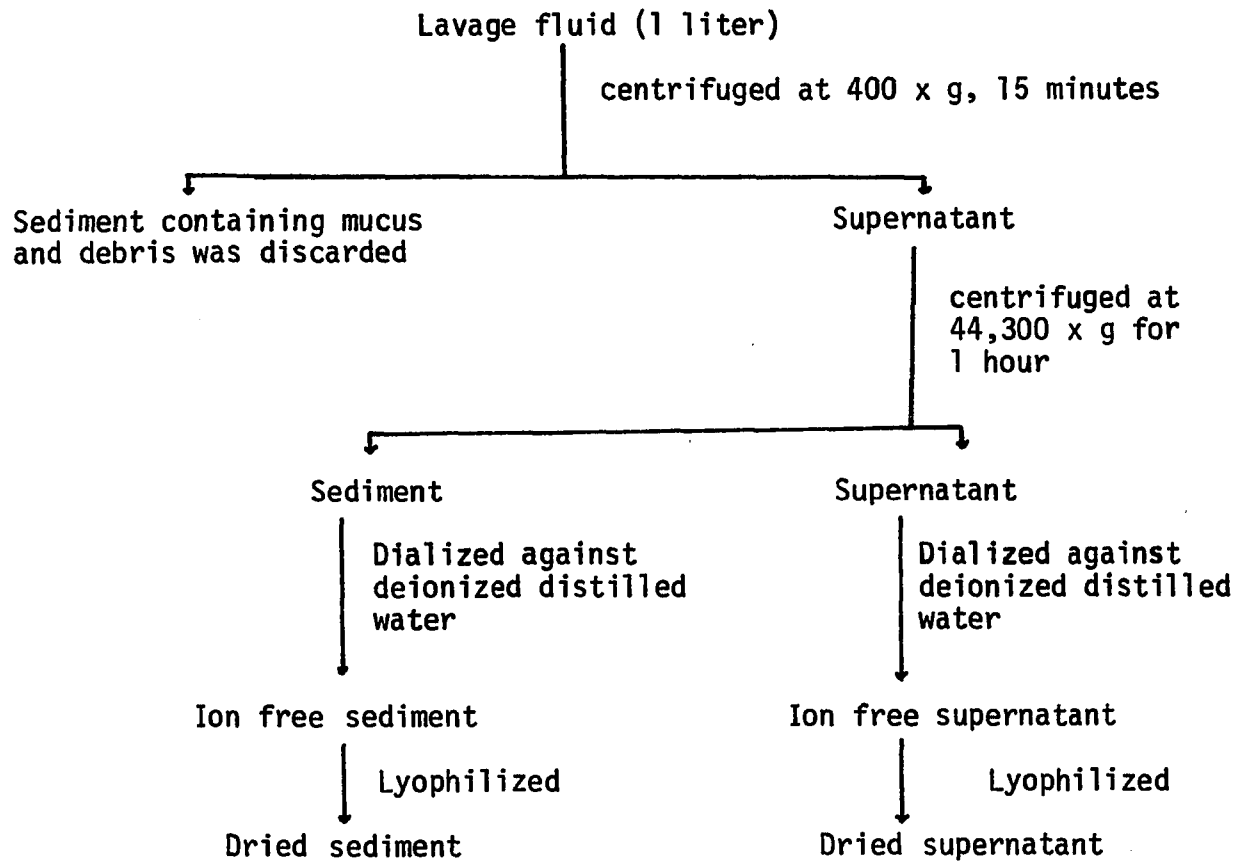
Lyophilized

Dried supernatant

Figure 2. Diagram of the separation of sediment and supernatant.

disrupted lung tissues were lyophilized and the samples were kept in the freezer at -30°C for further analysis.

Light Microscopy

The small pieces of lung tissue were fixed in 10% buffered formalin for at least 10 days, dehydrated in ethanol, cleared in chloroform, and embedded in paraffin. Tissues were sectioned and stained with hematoxylin-eosin. This stain was useful for identification of the lesion areas. The slides were examined and photographed.

The Protocol for Experiment 2

The pigs were divided into two groups with examination of the first group at the fourth week and second at the fifth week post inoculation with M. hyopneumoniae (Figure 3).

The Procedure for Studies of ^{14}C Incorporated into Phospholipid Synthesis in the Lung

Palmitic acid-^{14}C, specific activity 750 mci/mmole, molecular weight 256.4 was purchased from New England Nuclear Corp., Boston, Mass. All carbons were labeled:

$$*CH_3(*CH_2)_{14}*COOH$$
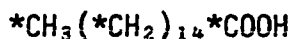
The radiochemical purity of palmitic acid-^{14}C was greater than 98 percent. The palmitic acid-^{14}C with a total radioactivity of 300 uci was dissolved in 10 ml of 0.00012 N NaOH to give a stock solution of sodium palmitate containing 300 uci/10 ml. Prior to its use, this solution was

diluted one in ten with 5% bovine albumin solution. The final solution of 100 ml contained ^{14}C radioactivity 300 uci.

The Administration of Sodium Palmitate-^{14}C into Animals

The studies of the incorporation of palmitate-^{14}C into phospholipid of pulmonary surfactant and lung tissue were performed in young pigs at the fourth and fifth week post infection with Mycoplasma hyopneumoniae. Both infected and control animals were anesthetized with 30 mg/kg intravenous sodium pentobarbital. The palmitate-^{14}C 1 uci/kg was injected via femoral vein. At 5 hours after intravenous injection of palmitate-^{14}C the animal was killed to collect lavage fluid, blood, serum, and lung tissue. The methods of collecting these samples were similar to the Experiment 1 as described previously.

The Counting of ^{14}C Radioactivity

One ml of serum was hydrolized with 10 ml of instagel[1] for overnight and then was ready for ^{14}C counting. The individual phospholipid spots from their layer chromatography plates were mixed with 10 ml instagel.

Twenty mg of dried sedimented pulmonary surfactant and lung tissue were hydrolized with 1 ml of toluene-250[1] at room temperature for an hour with intermittent shaking. The clear solution was diluted to 10 ml with fluor solution, containing 0.1 gm of POPOP (1,4-bis-2-(4-methyl-5-phenyl-

---

[1]Instagel, Packard Instrument Co., Downers Grove, Illinois.

Pigs inoculated
Mycoplasma hyopneumoniae

	Period 1 - 4 weeks post inoculation	Period 2 - 5 weeks post inoculation
Number of treated animals	4	4
Number of control animals	4	4
Symptomatic and gross observation	+	+
Blood chemistry	+	+
Phospholipid synthesis studies used ^{14}C	+	+
Lung lavage	+	+

Figure 3. Outline of the general plan of study in Experiment 2.

oxazolyl)-benezene) and 5 gm of PPO (2,5-diphenyloxazole), per liter of toluene.

Radioactivity measurements were made using a three channel Tricarb liquid scintillation spectrometer (model 3375).[1] The labelled ^{14}C in each vial was counted for a 10 minute period. Quench corrections were made with an external ^{137}Cs reference source and a quenched sample series appropriate for the range of quenching in the samples. Counting efficiency for ^{14}C in blood, sediment, lung tissue samples were of the order of 64.77±0.50, 81.46±0.25, 79.47±0.17 percent, respectively. Standards with known quench values were included with all counting runs to insure correct quench corrections.

Phospholipid Analysis

Lipid extract

The freeze-dried samples of pulmonary surfactant and lung tissue were extracted twice with chloroform:methanol (2:1, V/V). The volume of extracted lipids was reduced by warming the extract in water bath to 38°C and evaporating the solvents under nitrogen. The samples were redissolved in 2 ml of chloroform:methanol (1:1, V/V).

Phospholipid fractionation

Fifty microliters of each lipid extract sample were applied with a micropipette to silicon gel Prekote absorbent plates[2] along a transverse

---

[1]Spectrometer, Packard Instrument Co., Downers Grove, Illinois.

[2]Adsorbosil TLC plate, Applied Science Lab., Inc., Pennsylvania.

63

line 2.5 to 3.0 cm from the bottom. Application of 50 micrograms of the
reference mixture containing dipalmitoyl-L-α-lecithin, phosphatidyl
ethanolamine, sphingomyelin and lysolecithin[1] was made to the same plate.

The development of the chromatogram of phospholipid references and
unknowns was performed at room temperature using chloroform:methanol:
water, 80:25:4 (V/V). The chromatographic chambers were prepared 20
minutes prior to insertion of the plates. The chromatogram was allowed to
develop until the solvent front reached 18 cm from the origin; this re-
quired about 1 hour. The phospholipids were identified by iodine
vaporization in a dessicator.

The Microdetermination of Phosphorus

The samples of alveolar surfactant, lung tissue, and the spots of
phospholipid from thin layer chromatography were analyzed for phosphorus
as described by Morrison (1964).

The Gravimetric Determination for Total Lipid

The gravimetric determinations were made with 1 ml aliquot of lipid
extract. The samples were evaporated in hand-shaped aluminum foil cups
brought to constant weight. The cups were heated at 90°C for 20 minutes,
transferred to a dessicator and weighed when cool.

---

[1] Standard phospholipids, Sigma Chemical Co., St. Louis, Missouri.

Fatty Acid Methylation

The methylation was carried out by the method of Morrison and Smith (1964) using Boron trifluoride as the catalyst and MeOH as the trans-methylating agent. Total lipid extracts were methylated by using 1 ml aliquants. The samples were evaporated to dryness under nitrogen in Teflon-lined screw cap culture tubes, and then were taken up in 1 ml of methylating solvent mix (14% BF_3 in MeOH, using 1 ml of reagent for 4-16 mg of lipid). The tube was heated in boiling water for 2 minutes. After methylation, the samples were cooled and treated with two volumes of Pentane and one volume of water, mixed briefly and centrifuged until the layers were clear. The Pentane layer, containing the fatty acid methyl esters, was concentrated for injection into a gas liquid chromatograph.

Gas Liquid Chromatography

Fatty acid methyl esters were analyzed using a Beckman GC-72-5 chromatograph.[1] The instrument is equipped with dual flame ionization detectors. Conditions for the analysis were as follows: stainless steel column 10' by 1/8", packed with 3% ethylene glycol succinate on gas-chrom. G100/120 mesh HP; column temperature 180°C, injection temperature 200°C, detector line temperature 210°C, detector temperature 250°C, carrier gas flow 30 ml/min.

The commercial standards of fatty acids were obtained from Applied Science Lab. and the uncorrected peak areas were determined using an

---

[1]Beckman Co., Fullerton, California.

Infotronics Automatic Digital Integrator Model CRS-208.[1] The relative
percentage of each fatty acid component was calculated.

The Determination of Protein Content

Each sample of pulmonary surfactant sediment, pulmonary supernatant
sediment and lung tissue was analyzed for percent of protein content by
the method of Lowry et al. (1954). Bovine serum albumin was used as a
standard.

Statistical Analysis

In Experiment 1 data was collected at the 4th, 5th or 6th week post
inoculation with M. hyopneumoniae. In Experiment 2 data was obtained only
at the 4th and 5th week post inoculation. Both experiments were designed
as a two way analysis of variance (Ostle and Mensing, 1975).

Analysis of variance table for Experiment 1.

SV	DF	SS	MS	F
Total	31			
Treatment	1			
Period	2			
Treatment x period	2			
Error	26			

---

[1]Infotronics Corporation, Austin, Texas.

Analysis of variance table for Experiment 2

SV	DF	SS	MS	F
Total	15			
Treatment	1			
Period	1			
Treatment x period	1			
Error	12			

The student t test was used for the comparison between the control and treated animals at each time period. The means of the percentages of the following factors were analyzed:
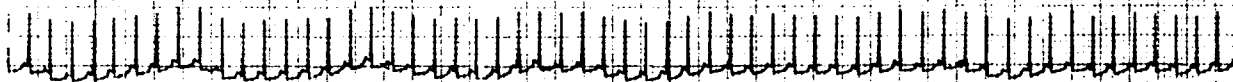
1. Fatty acids of sedimented surfactant.

2. Plasma protein fractions.

3. Differential white blood cell count.

4. Phospholipid composition of the lung and sedimented surfactant.

5. The distribution of palmitate-^{14}C in phospholipid fractions of lung and sedimented surfactant.

Figure 5. Recorder output.

PAPER SPEED = 1 cm/sec.

CHANNEL ONE: EKG (LEAD II)

CHANNEL TWO: ESOPHAGEAL PRESSURE   Esophageal pressure = 4 mmHg
                1 mm pen deflection = 1 mmHg

                                                          4
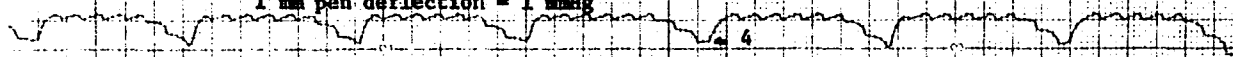
CHANNEL THREE: VELOCITY   Expiratory Velocity = 22 L/min.

                                    T   Expiratory
                                    11
                                     5   Inspiratory
                                     2   Inspiratory Velocity = 10 L/min.
        1 mm pen deflection = 2 L/min flow
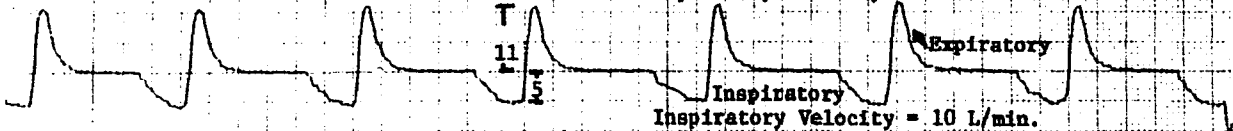
CHANNEL FOUR: TIDAL VOLUME   Tidal volume = 118 ml.

                                    T   Expiratory
                                    9
                                    1
                                                      Inspiratory
        1 mm pen deflection = 13.1 ml volume
        RESPIRATORY RATE = 18 breaths/minute

63
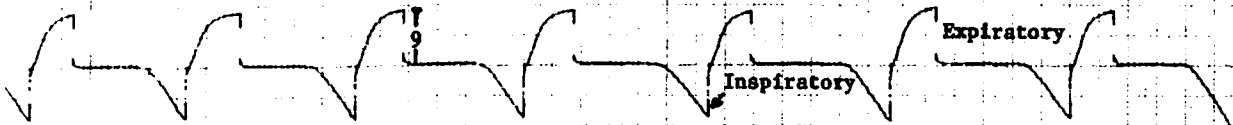
Figure 6.   X-Y respiratory loop studies.

PRESSURE-VOLUME LOOP

x axis (pressure); 1 mm = 0.67 mmHg
y axis (volume); 1 mm = 10 ml V_T

Dynamic lung compliance

$$= \frac{ml \; V_T}{cm \; H_2O}$$

$$= \frac{170}{11 \; mm \; x \; .67 \; mmHg/mm \; x \; 1.36 \; cm \; H_2O/mmHg}$$

$$= 16.97 \; ml/cm \; H_2O$$

FLOW-VOLUME LOOP
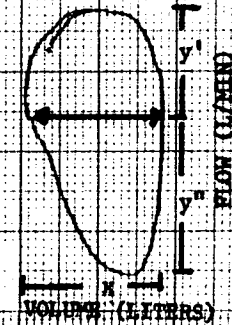
y axis (flow); 1 mm = 1.1 L/min.
x axis (volume); 1 mm = 10 ml.

y' = inspiratory velocity
y" = expiratory velocity
x = tidal volume

V_T = 170 ml.
Expiratory velocity = 22 L/min.
Inspiratory velocity = 14.3 L/min.

PRESSURE-FLOW LOOP
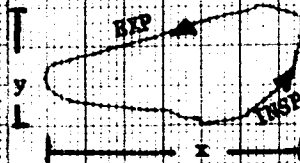
y axis (pressure); 1 mm = 0.67 mmHg
x axis (flow); 1 mm 0.9 L/min.

$$Air-Resistance = \frac{15 \; mmHg \; x \; 0.67 \; mm/mmHg \; x \; 1.36 \; cm \; H_2O}{33 \; x \; 0.9 \; L/min/60 \; sec/min.}$$

$$= 27.61 \; cm \; H_2O/L/sec.$$

RESULTS

Experimental Design

Experiment 1

This experiment was a study of physiopathogenesis. Thirty-two specific-pathogen-free (SPF) pigs, 19 experimental and 13 control animals, were used. The experimental animals were inoculated intratracheally with M. hyopneumoniae. Physiological and post mortem studies were conducted on both experimental and control pigs at the 4th, 5th and 6th week post inoculation.

Experiment 2

This experiment was designed to study the effect of M. hyopneumoniae infection on phospholipid synthesis. It involved a total of 16 specific-pathogen-free pigs, 8 experimental and 8 control animals. The experimental pigs were inoculated intratracheally with M. hyopneumoniae. The studies were performed at 4th and 5th week post inoculation.

Clinical Signs

The only clinical sign of infection that was observed with the inoculated pigs was coughing. Coughing did not occur in all infected pigs. When it did occur coughing appeared early, frequently at about two weeks after inoculation, in the infected group and was prolonged until the 6th week of the experiment. However, the infected pigs showed normal behavior of eating and drinking and normal general appearance. The

respiratory movements of inoculated pigs were normal. No nasal discharge was evident and the body temperature was normal.

Gross Lesion

Gross pneumonic lesions had already developed as early as 4th week of infection. The lesions were frequently confined to the apical, cardiac, and intermediate lobes, and the upper part of diaphragmatic lobe. The purple to grey colored areas of consolidation were demarcated clearly from the normal lung tissue. There were signs of edema throughout the lung but the diaphragmatic lobes were especially edematous. All of the inoculated pigs showed the gross pneumonic lesions (see Figures 7 and 9). There were no lesions in the lung of control pigs.

Light Microscopy

There was hyperplasia of peribronchiolar and perivascular lymphoid tissue, alveolar interstitial thickening, accumulation of the leukocytes and cellular debris in the lumen of the bronchi, and distension of the peribronchial glands with cellular exudate. The leukocytes extensively invaded the alveoli. The muscularis mucosa was ruptured and catarrh of bronchial cells occurred (see Figures 9, 10, 11, 12, 13, 14).

Serology

The microtitration complement-fixation test was performed on serum samples from both control pigs and pigs intratracheally inoculated with M. hyopneumoniae. At the 4th week of infection sera from the infected

Figure 7. Gross pneumonic lesion from a pig infected intratracheally
with broth culture of M. hyopneumoniae at 6th week post
inoculation.

Figure 8. Gross pneumonic lesion from a pig infected intratracheally
with broth culture of M. hyopneumoniae at 6th week post
inoculation.
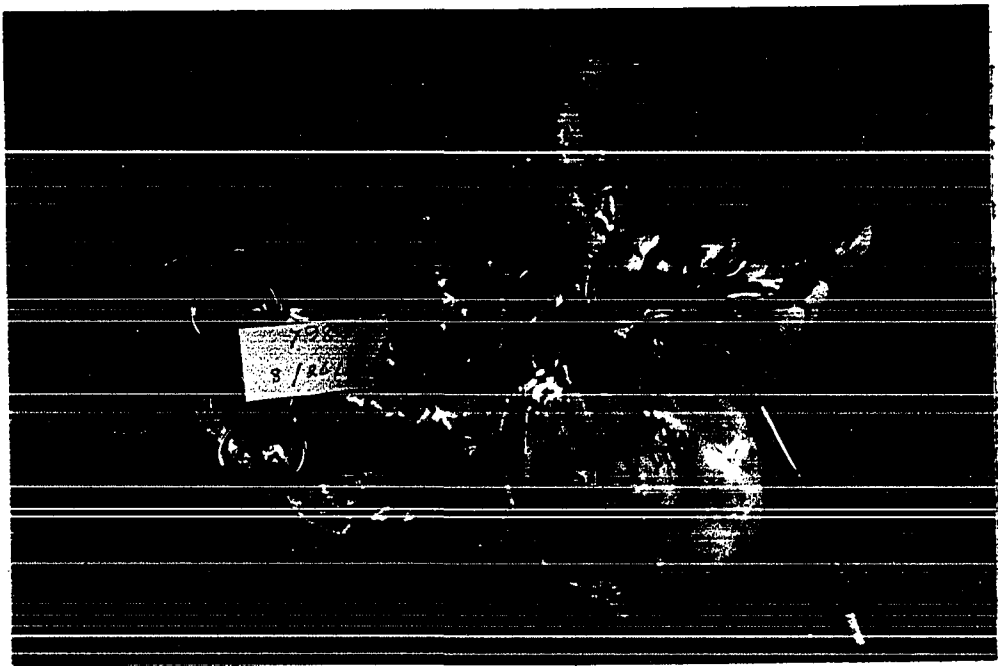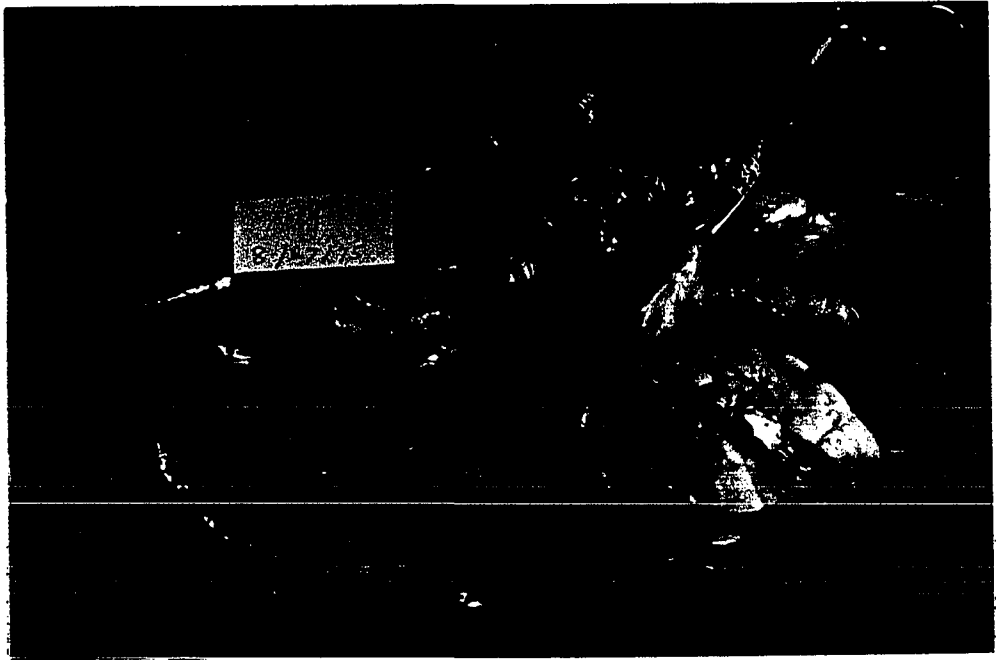
Figure 9. The tertiary bronchiole and alveoli in control pigs.
Hematoxylin and eosin stain, 158X.

Figure 10. The secondary bronchiole and alveoli in control pigs.
There is no lesion. Hematoxylin and eosin stain, 394X.
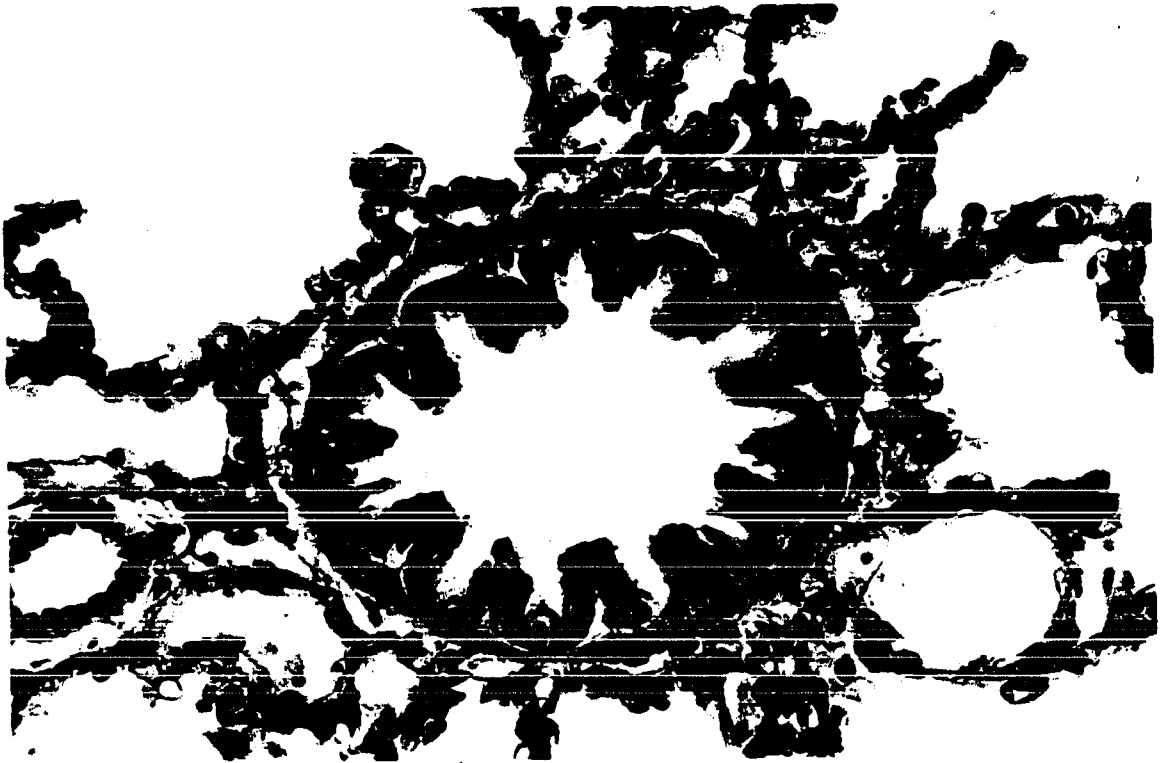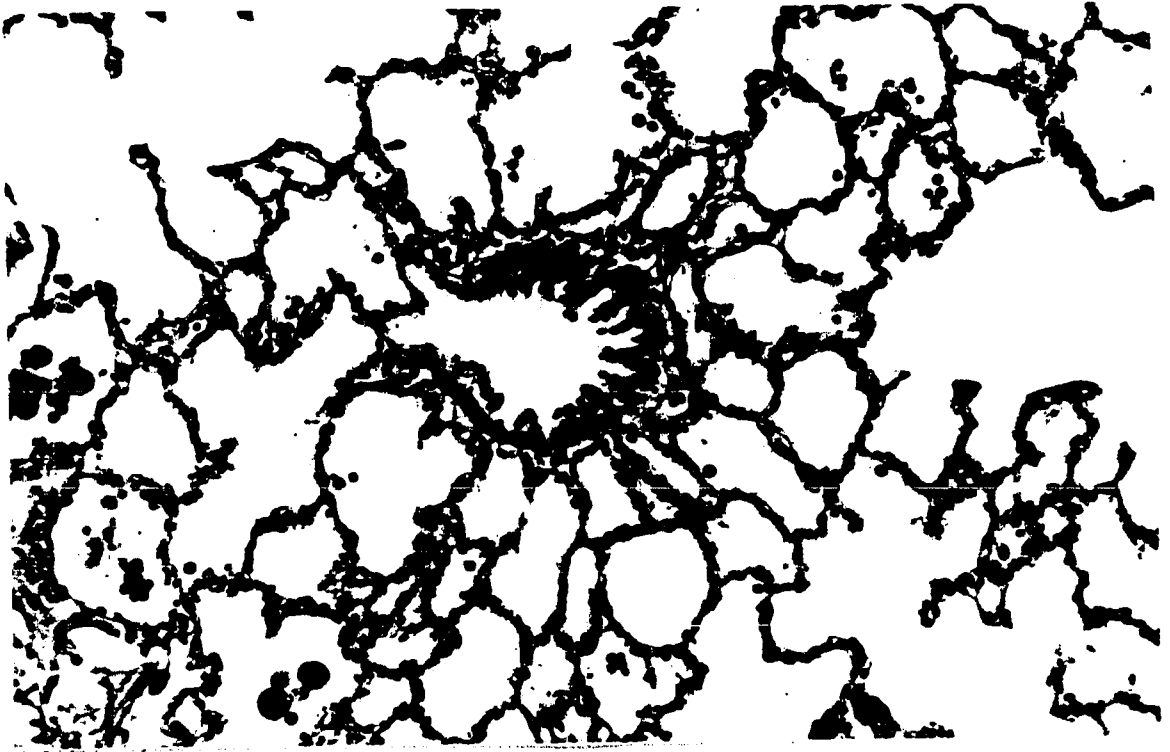
Figure 11. Lung of pig 6 weeks after M. hyopneumoniae intratracheally
            exposure. The bronchus contains leukocytes and cellular
            debris. Leukocytes already infiltrated the alveoli. Hema-
            toxylin and eosin stain, 158X.

Figure 12. Peribronchial glands were distended with cellular exudate,
            leukocytes, and cellular debris filled the lumen of the
            bronchus. Hematoxylin and eosin stain, 158X.
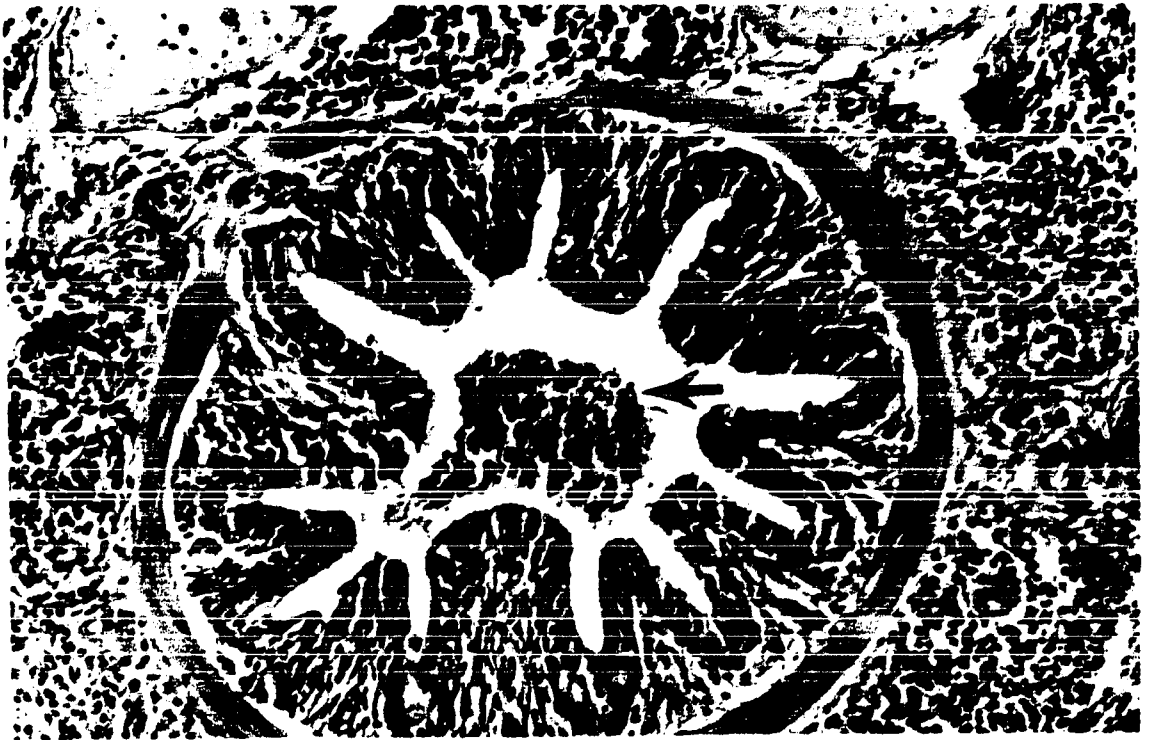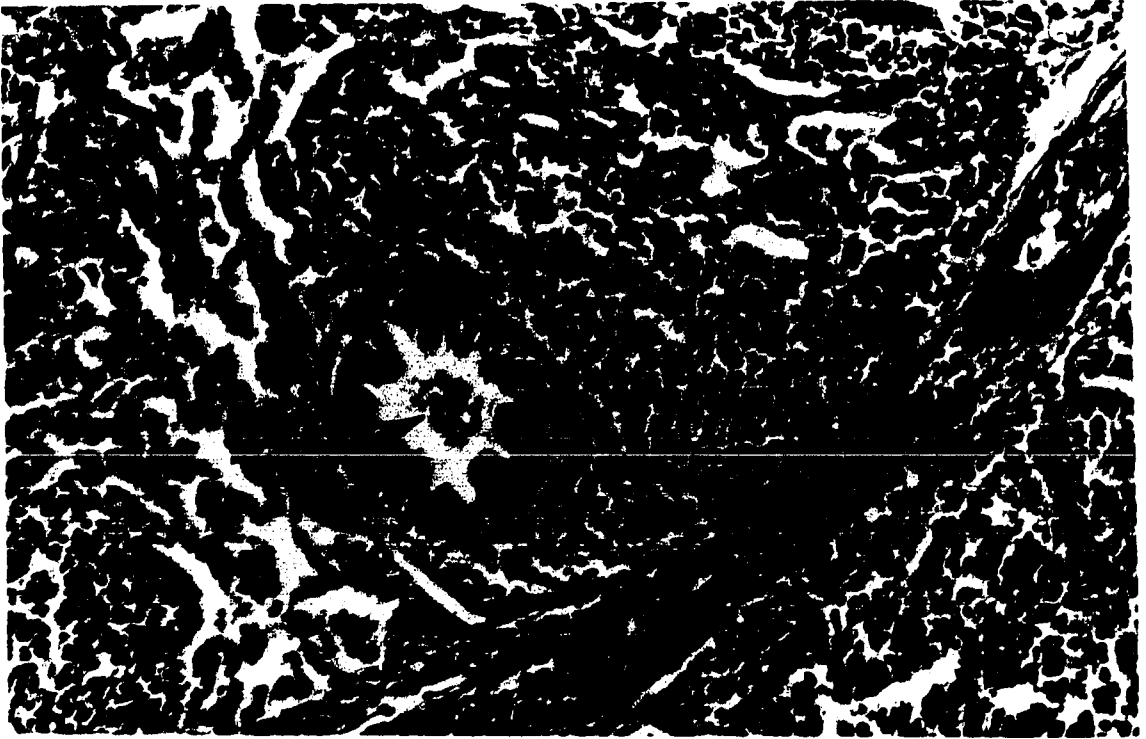
Figure 13. Lung section of an inoculated pig showing peribronchiolar lymphoid hyperplasia (arrow), bronchus containing leukocytes and cellular debris, and edematous area, at 6th week post infection. Hematoxylin and eosin stain, 158X.

Figure 14. Lung section of an inoculated pig at 6th week post infection demonstrated invasion of alveoli by leukocytes, rupture of muscularis mucosa (arrow) of bronchus, and catarrhal of bronchus cells. Hematoxylin and eosin stain, 158X.
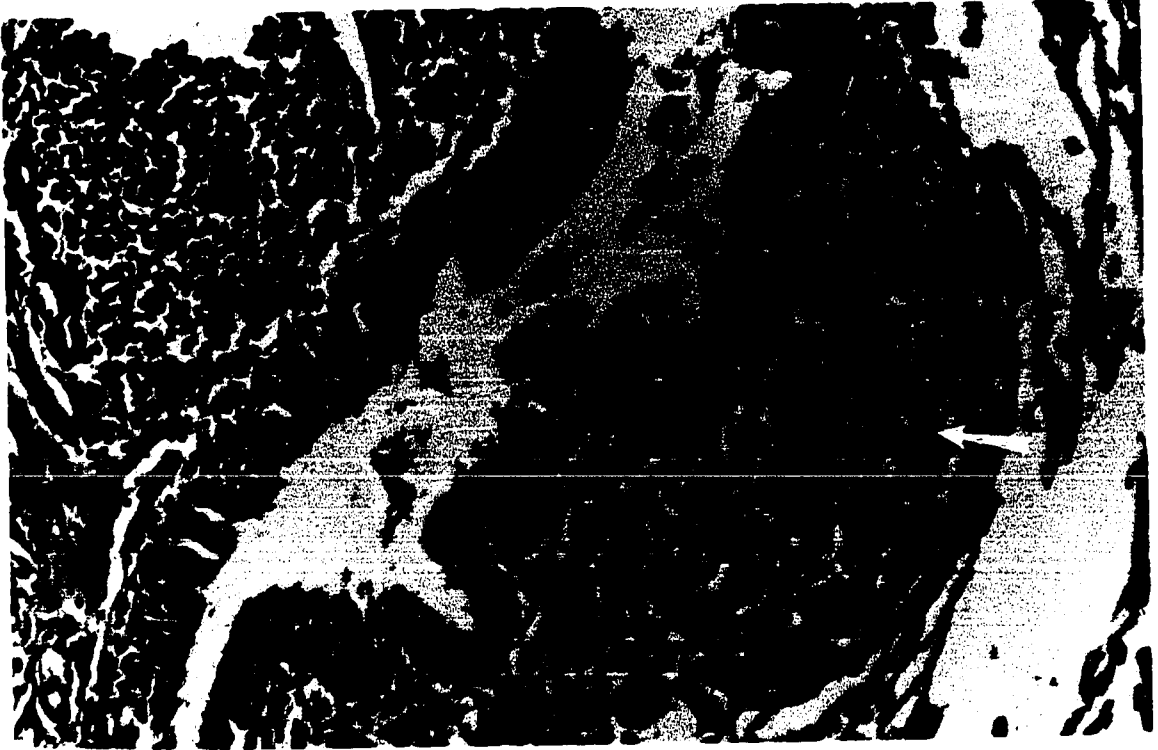
pigs had antibody titers between 1:16 to 1:64, with most of the sera from infected pigs showing a titer of 1:32. The titers of sera from the pigs at the 5th week of infection were between 1:16 to 1:64, with a titer of 1:64 dominant among the animals of the group. The antibody titers at the 6th week post infection were between 1:10 and 1:32, with 1:32 as the most prevalent titer.

For Experiment 2 sera from most of the infected animals showed an antibody titer of 1:8 and 1:32 for the 4th week and 5th week of infection, respectively (Table 1).

Body Weight Gain

At the start of the experiment, the pigs of the control and treatment groups were uniform in body weight. For the control group, there was no statistical difference in the rate of body weight gain among the pigs in the three periods of trial. The infected pigs showed a marked decrease in rate of body weight gain in each of three periods. The body weight gain per day decreased by 9.50, 7.31, and 14.67 percent of that of the control pigs at 4th, 5th and 6th week of infection, respectively (Table 2). There was a significant difference ($P<0.01$) of body weight gain among pigs in the three periods of experiment.

Body Temperature, O_2 Consumption, Heart Rate
and Respiratory Rate

The control and the infected animals showed a normal body temperature of about 102.2°F for all periods. There were little changes in the heart

Table 1. CF titer of antibodies against M. hyopneumoniae in pigs inoculated by intratracheal injection

	Experiment 1			Experiment 2	
Pig No.	Week post inoculation	Titer	Pig No.	Week post inoculation	Titer
913	4	1:32	933	4	1:16
908	4	1:16	934	4	1:8
916	4	1:32	935	4	1:8
914	4	1:64	936	4	1:8
917	4	1:32			
786	4	1:32			
912	5	1:64	931	5	1:32
915	5	1:64	938	5	1:16
791	5	1:32	932	5	1:32
921	5	1:32	937	5	1:32
919	5	1:64			
906	5	1:16			
798	6	1:16			
794	6	1:32			
800	6	1:32			
795	6	1:16			
922	6	1:32			
923	6	1:32			
905	6	1:32			

Table 2. Body weight, and body weight gain per day of control and experimental pigs

	Initial Body wt (kg)	Body wt gain* (gm/day)
4th week of infection		
Control (4)[a]	35.68±2.16[b]	657.95±30.23
Infected (6)	35.11±1.44	595.46±18.45
5th week of infection		
Control (4)	32.73±1.54	564.78±25.40
Infected (6)	31.90±1.10	523.49±33.18
6th week of infection		
Control (5)	32.64±2.04	476.36±31.73
Infected (7)	27.53±1.24	406.50±24.07

[a]Number of observations.

[b]Values are mean±SEM.

*Difference among 3 periods was significant at P<0.05.

rates and respiratory rates. The mean values of heart rate varied from 138 to 194 beats per minute and respiratory rates varied from 18 to 22 breaths per minute. The infected pigs showed a highly significant decrease (P<0.01) in oxygen consumption. The control pigs consumed 8.78, 9.25 and 9.46 ml/min/kg of oxygen while the infected pigs consumed 6.98, 7.48 and 7.54 ml/min/kg of oxygen at 4th, 5th and 6th week post infection, respectively (Table 3).

Table 3. Body temperature, O_2 consumption, heart rate, and respiratory rate of control and infected pigs

	Body temp. (°F)	O_2 consumption** (ml/min/kg)	HR (beat/min)	RR (Breaths/min)
4th week of infection				
Control (4)[a]	102.1±0.45[b]	8.78±0.33	159±11	21±1.0
Infected (6)	102.1±0.33	6.98±0.37	138±5	18±0.5
5th week of infection				
Control (4)	102.2±0.14	9.25±0.24	183±15	20±1.0
Infected (6)	102.4±0.20	7.48±0.14	194±14	22±2.0
6th week of infection				
Control (5)	102.6±0.17	9.46±0.58	174±13	18±0.4
Infected (7)	102.2±0.30	7.54±0.32	178±11	19±0.9

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at $P<0.01$.

The Change of Respiratory Parameters

There were the significant increases (P<0.01) in tidal volume in in-
fected animals. The inspiratory flow rate was also increased in the in-
fected pigs but failed to show the significant difference from control
pigs. There were significant increases (P<0.01) in expiratory flow rate,
minute volume, and alveolar ventilation in the infected pigs (Table 4).
Most likely there were drastic changes in the mechanics of breathing and
these effects extended through all three periods of infection. The nega-
tive esophageal pressure of infected pigs was increased to twice
that of the control pigs, which is a significant difference at P<0.01.
The dynamic lung compliance decreased in infected pigs (P<0.07). The air-
way resistance of infected pigs showed a marked increase over that of the
control pigs. The work of breathing was increased significantly in in-
fected pigs (P<0.01) both when work was compared as gm-m/kg and when it
was compared as gm=m/min/kg (Table 5).

Arterial pH, PaO_2, $PaCO_2$, HCO_3^-, Base Excess, P_AO_2, P_ACO_2

The pH of arterial blood ranged between 7.35 and 7.37 for control
pigs and between 7.31 and 7.33 for the infected pigs. The PaO_2 varied
between 72 and 75 mmHg for control animals, while that for the infected
pigs ranged between 66 and 68 mmHg. The mean values of $PaCO_2$ in control
pigs ranged from 46 to 49 mmHg. The infected pigs showed a slight de-
crease with $PaCO_2$ varying between 42 and 45 mmHg. The decreases in
arterial HCO_3^- of infected pigs were correlated with the decreases in $PaCO_2$
(Table 6). There were highly significant decreases of base excess (BE)

Table 4. Tidal volume, inspiratory and expiratory flow rate, minute volume, and alveolar ventilation of control and infected pigs

	Tidal volume** (ml)	Inspiratory flow (L/min)	Expiratory flow** (L/min)	Minute volume** (ml)	Alveolar ventilation** (ml)
4th week of infection					
Control (4)[a]	158±5[b]	13.75±0.80	20.45±0.69	3342±90	1675±43
Infected (6)	223±9	20.42±2.12	30.67±0.72	4029±249	2633±184
5th week of infection					
Control (4)	179±3	15.10±1.00	21.63±1.43	3617±257	2166±210
Infected (6)	222±8	16.83±1.50	28.00±1.24	4870±396	3448±242
6th week of infection					
Control (5)	177±4	14.74±0.85	21.96±1.25	3266±146	1945±123
Infected (7)	255±9	20.00±2.37	27.43±1.36	4879±287	3721±247

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at P<0.01.

Table 5. Esophageal pressure, dynamic lung compliance, airway resistance, work of breathing, of control and infected pigs

	P_E** (cm H_2O)	C_{dyn} (ml/cm H_2O)	R_A (cm H_2O/L/sec)	Work of breathing** (gm-m/kg)	Work of breathing** (gm-m/kg)
4th week of infection					
Control (4)[a]	-6.46±0.81[b]	25.36±2.42	18.82±1.84	0.03±0.01	0.60±0.06
Infected (6)	-12.13±0.79	19.03±1.99	23.77±1.60	0.08±0.01	1.39±0.09
5th week of infection					
Control (4)	-6.97±0.58	26.30±2.67	19.81±2.57	0.04±0.00	0.79±0.11
Infected (6)	-11.79±1.20	19.76±1.85	25.71±3.13	0.08±0.01	1.75±0.13
6th week of infection					
Control (5)	-7.48±0.68	24.40±2.09	21.10±2.94	0.04±0.01	0.77±0.11
Infected (7)	-14.09±0.90	18.45±1.18	31.08±2.06	0.13±0.01	2.53±0.27

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at P<0.01.

Table 6. The measurement of blood pH, PaO_2, $PaCO_2$, HCO_3^-, BE, CO_2 concentration and alveolar O_2, alveolar CO_2 in control and infected pigs

	pH	PaO₂ (mmHg)	PaCO₂ (mmHg)	HCO₃⁻ (mEq/L)	BE** (mEq/L)	CO₂ conc. (mEq/L)	PAO₂ (mmHg)	PACO₂* (mmHg)
4th week of infection								
Control (4)[a]	7.35±0.03[b]	75±4	46±4	24.80±1.00	0.10±1.39	26.25±1.07	101±2	40±1
Infected (6)	7.31±0.04	67±2	42±1	20.87±2.24	-4.35±0.77	22.13±2.09	112±3	32±2
5th week of infection								
Control (4)	7.35±0.04	72±6	47±4	26.13±3.16	1.63±2.70	27.63±3.25	114±3	34±2
Infected (6)	7.32±0.04	66±1	43±2	21.95±1.34	-3.50±1.80	23.23±1.35	112±3	31±2
6th week of infection								
Control (5)	7.37±0.01	75±1	49±1	27.24±0.73	3.08±0.39	29.24±0.53	93±6	45±3
Infected (7)	7.33±0.02	68±5	45±1	22.99±0.55	-2.31±0.74	24.39±0.56	109±4	33±2

[a]Number of observations.

[b]Values are mean±SEM.

*Difference between control and infected pigs was significant at P<0.05.

**Difference between control and infected pigs was significant at P<0.01.

in the infected pigs ($P<0.01$). The concentration of CO_2 in the arterial blood showed a decrease in infected pigs as correlated to PaCO (Table 6). The alveolar CO_2 partial pressure showed a significant decrease ($P<0.05$) in the infected animals while the alveolar O_2 partial pressure did not change (Table 6).

Hb, PCV, RBC and WBC

There were slight increases in Hb and PCV in the infected pigs. The increase in RBC counts tended to parallel the PCV values (Table 7). The mean values of total WBC in control pigs varied from 13.50×10^3 to 17.06×10^3 per Cmm. The counts were higher in infected pigs and varied between 18.23×10^3 and 19.81×10^3 per Cmm. However, there were no significant differences of Hb, PCV, RBC and WBC between the control and infected pigs (Table 7).

Differential Leukocyte Counts

The percent of neutrophils increased while the percent of lymphocytes decreased in the infected pigs. Monocyte and eosinophil number increased slightly in the infected pigs. The number of basophils increased in the infected pigs but these cells were nearly absent in control animals (Table 8).

Plasma Protein and Glycoprotein Fractions

There were a number of distinct bands of plasma protein as shown in the electrophoresed gels (Figures 15, 16, 17). These bands were

Table 7. Hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC) in control and infected pigs

	Hb (gm/100 ml)	PCV (%)	RBC # x 10^6/cmm	WBC # x 10^3/cmm
4th week of infection				
Control (4)[a]	11.83±0.68[b]	35±1	8.16±0.33	14.90±1.51
Infected (6)	12.52±0.49	37±2	7.83±0.26	18.43±1.28
5th week of infection				
Control (4)	10.53±0.29	32±1	7.05±0.15	13.50±1.39
Infected (6)	11.40±0.38	36±1	7.19±0.46	18.23±0.51
6th week of infection				
Control (5)	10.32±0.18	33±1	6.67±0.42	17.06±1.94
Infected (7)	10.53±0.26	33±1	6.95±0.56	19.81±0.60

[a]Number of observations.

[b]Values are mean±SEM.

classified into albumin, alpha (α_1, α_2), beta (β), and gamma (γ_1, γ_2). The increase of globulin fractions occurred from 4th week through 6th week of infection (Table B1). This resulted from significant increase in gamma globulin levels in the infected pigs. Alpha 1, alpha 2, beta globulin levels also showed slight increases in the infected animals (Figure 18). There were significant increases ($P<0.01$) in the total plasma protein in all infected pigs (Table B1).

The plasma glycoproteins appeared on only one band in both control and infected pigs (Figures 19, 20 and 21). A marked increase in density

Table 8. The percent of differential white blood cells in control and experimental pigs

	Neutrophil %	Lymphocyte %	Monocyte %	Eosinophil %	Basophil %
4th week of infection					
Control (4)[a]	28.50±0.65[b]	68.50±1.19	2.50±0.65	1.50±0.29	-
Infected (6)	31.33±6.47	62.50±5.63	3.67±0.61	2.20±0.58	1.67±0.67
5th week of infection					
Control (4)	26.75±6.49	67.75±6.84	4.25±1.10	1.50±0.50	1.00±0.00
Infected (6)	30.50±4.04	57.83±4.18	4.50±0.76	2.33±0.61	1.25±0.25
6th week of infection					
Control (5)	25.00±1.73	70.00±0.63	3.40±0.60	2.00±0.58	-
Infected (6)	33.14±5.95	59.57±5.75	4.43±0.61	1.29±0.29	1.00±0.00

[a]Number of observations.

[b]Values are mean±SEM.

Figure 15. The electrophoretic separation of plasma protein at 4th week of M. hyopneumoniae infection.

Figure 16. The electrophoretic separation of plasma protein at 5th week of M. hyopneumoniae infection.

Figure 17. The electrophoretic separation of plasma protein at 6th week of M. hyopneumoniae infection.
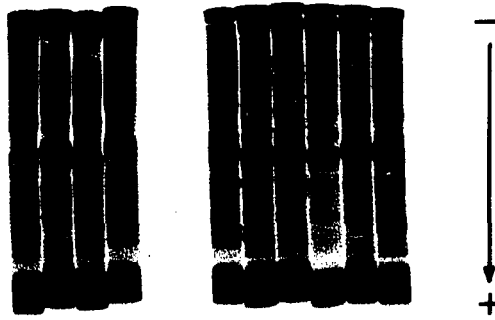
control pigs   infected pigs
Plasma protein (2 ul))
4th week of infection

Plasma protein (3 ul)
5th week of infection

control pigs   infected pigs
Plasma protein (3 ul)
6th week of infection
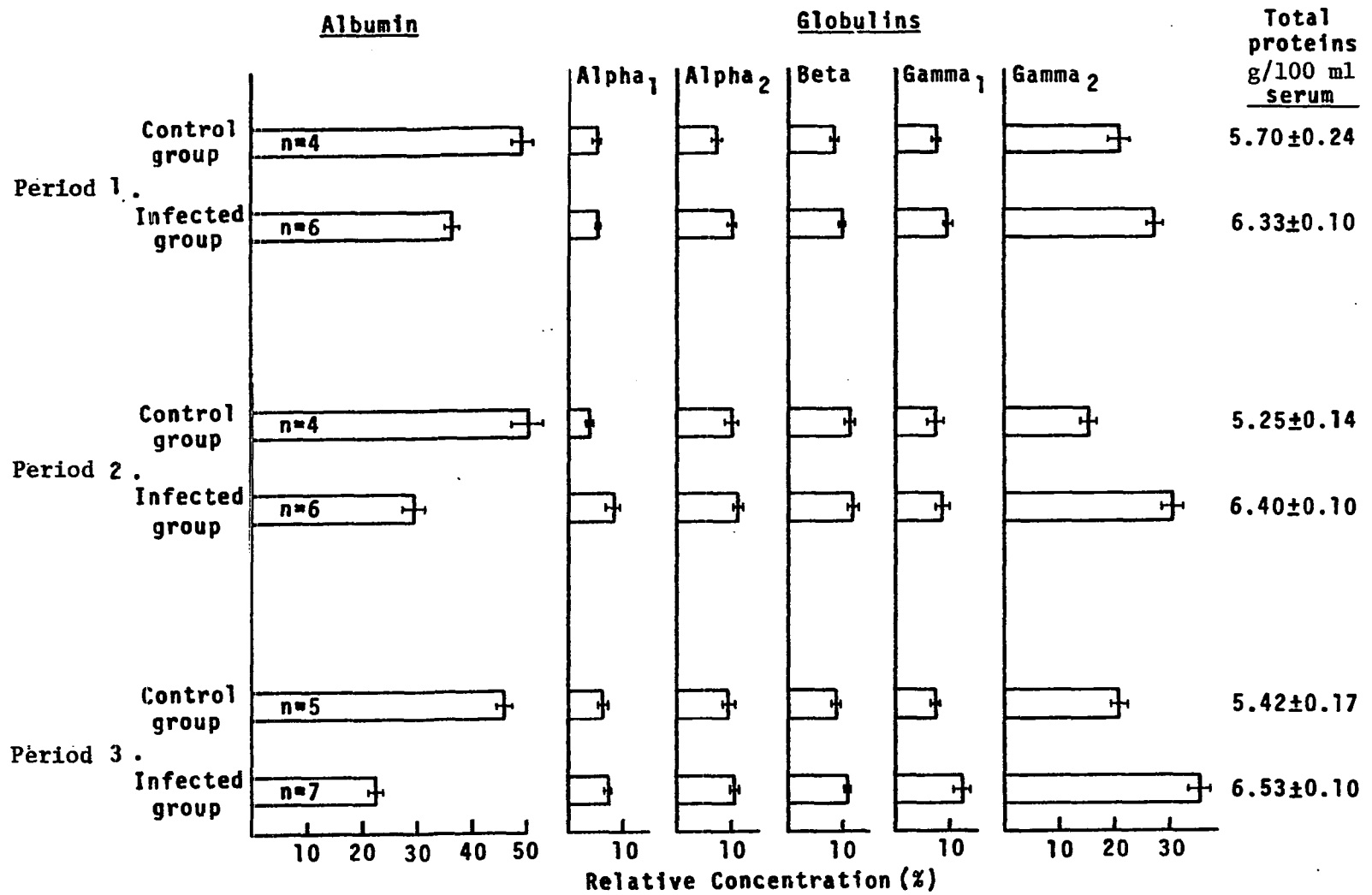
Figure 18. Mean values of plasma protein fractions and total proteins in the experimental and control groups of 4th, 5th, and 6th week post infection of *M. hyopneumoniae*.

Figure 19. Electrophoretic separation of plasma glycoprotein at 4th
week of M. hyopneumoniae infection.

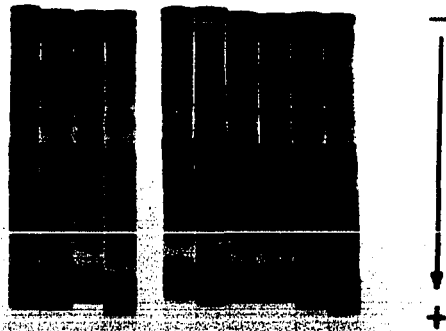Figure 20. Electrophoretic separation of plasma glycoprotein at 5th
week of M. hyopneumoniae infection.

Figure 21. Electrophoretic separation of plasma glycoprotein at 6th
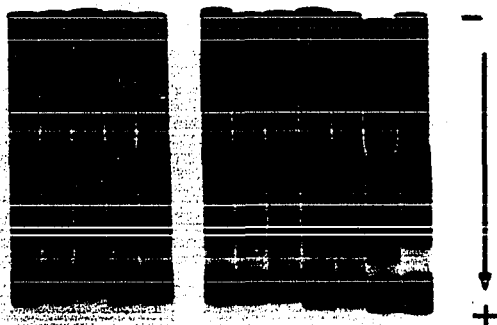week of M. hyopneumoniae infection.

control pigs   infected pigs

Glycoprotein in plasma (30 μl)
5th week of infection

control pigs   infected pigs

Glycoprotein in plasma (30 μl)
6th week of infection
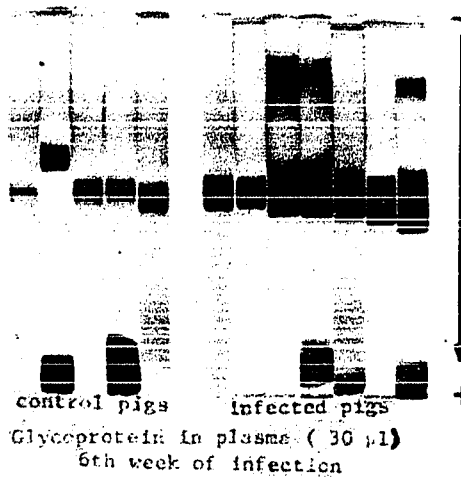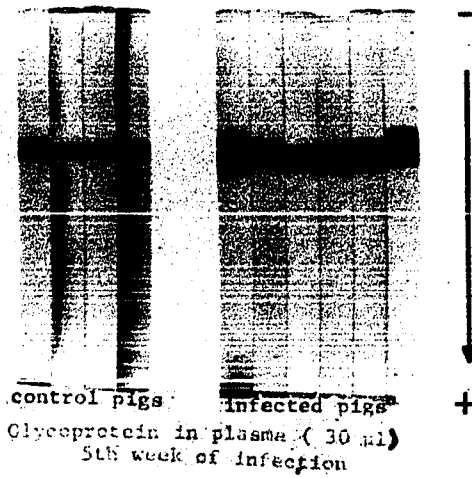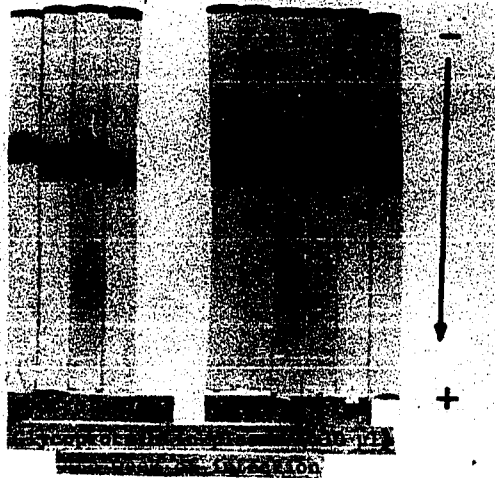
of glycoprotein band in infected plasma was noted. At the 6th week of infection, there appeared to be more than one band of glycoprotein in the electrophoregram in plasma from infected pigs (Figure 21).

Serum Electrolytes, Glucose, BUN, Cholesterol and
Lung Weight of Animals in Experiment 2

Concentrations of serum Na, K, Cl, glucose and cholesterol were somewhat variable among all animals. The mean values are shown in Table 9. There are no significant differences between the concentration of each substance for the control and that for the infected animals. The concentration of blood urea nitrogen (BUN) showed significant decreases ($P<0.01$) in the infected pigs. The lung weight of infected pigs per kg body weight was much greater than that of the control animals (Table 9).

Lavaged Materials from Lungs

The lavaged material from the lungs was collected in one liter of lavage fluid. This was separated into two parts by centrifugation, the sedimented surfactant and the supernatant fraction (Figures 22 and 23). The amount of sedimented surfactant and supernatant fraction were significantly increased ($P<0.01$) in infected pigs over that of control animals. The total amount of dried lavaged materials extracted from the lungs of control pigs varied from 228 to 266 mg per liter of lavage fluid, while that from the lungs of the infected pigs varied from 490 to 519 mg per liter of lavage fluid (Table 10).

Table 9. The serum Na, K, Cl, glucose, BUN, cholesterol and total lung weight of control and experimental pigs

	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Glucose (mg/100 ml)	BUN** (mg/100 ml)	Cholesterol (mg/100 ml)	Lung wt. (gm/kg body wt.)
4th week of infection							
Control (4)[a]	134.00 ±1.47[b]	5.95 ±0.12	99.75 ±0.50	96.75 ±2.69	13.50 ±0.87	103.00±1.29	9.62 ±0.14
Infected (4)	135.25 ±1.93	6.03 ±0.17	100.00 ±1.41	91.75 ±5.66	9.75 ±0.49	93.75±3.71	18.73 ±0.90
5th week of infection							
Control (4)	139.25 ±0.75	6.30 ±0.09	100.50 ±0.96	82.25 ±4.23	17.50 ±1.26	97.00±3.87	10.81 ±0.50
Infected (4)	135.75 ±1.31	5.78 ±0.22	99.75 ±0.48	89.50 ±5.98	10.25 ±0.63	96.50±2.22	19.49 ±6.0

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at $P<0.01$.

Figure 22. The general appearance of lyophilized sediment sur-
factant of control and infected pigs.

Figure 23. The general appearance of lyophilized supernatant fraction
of control and infected pigs.
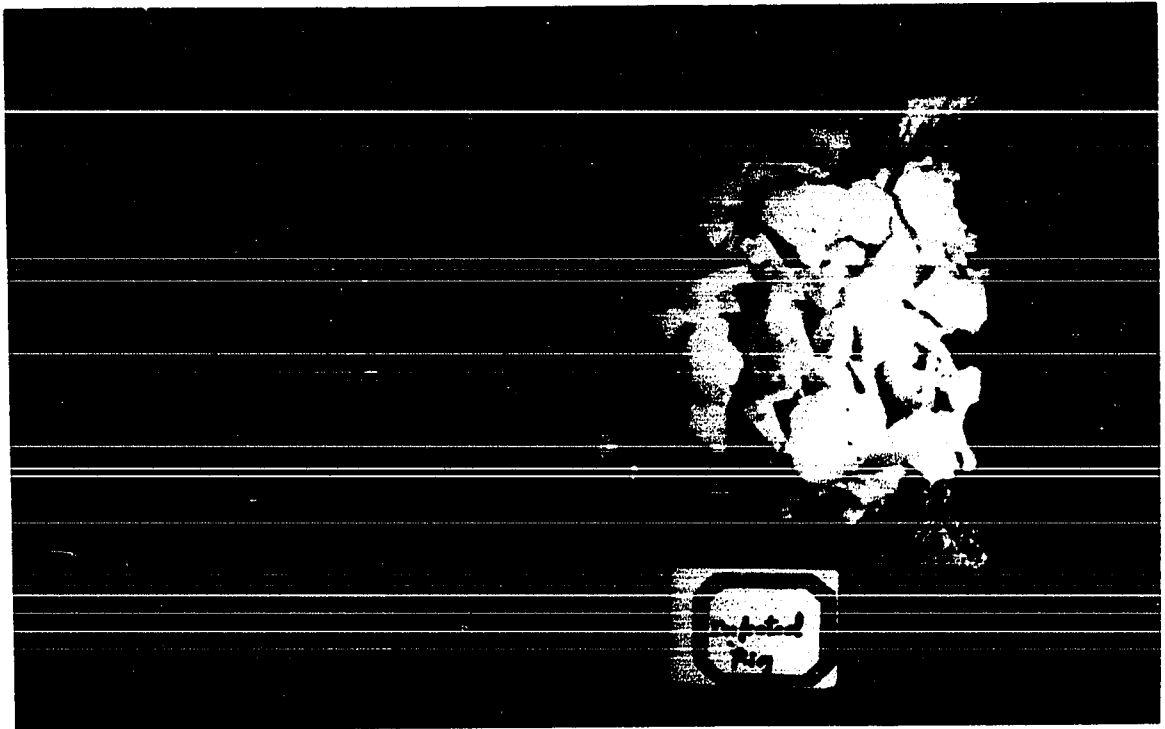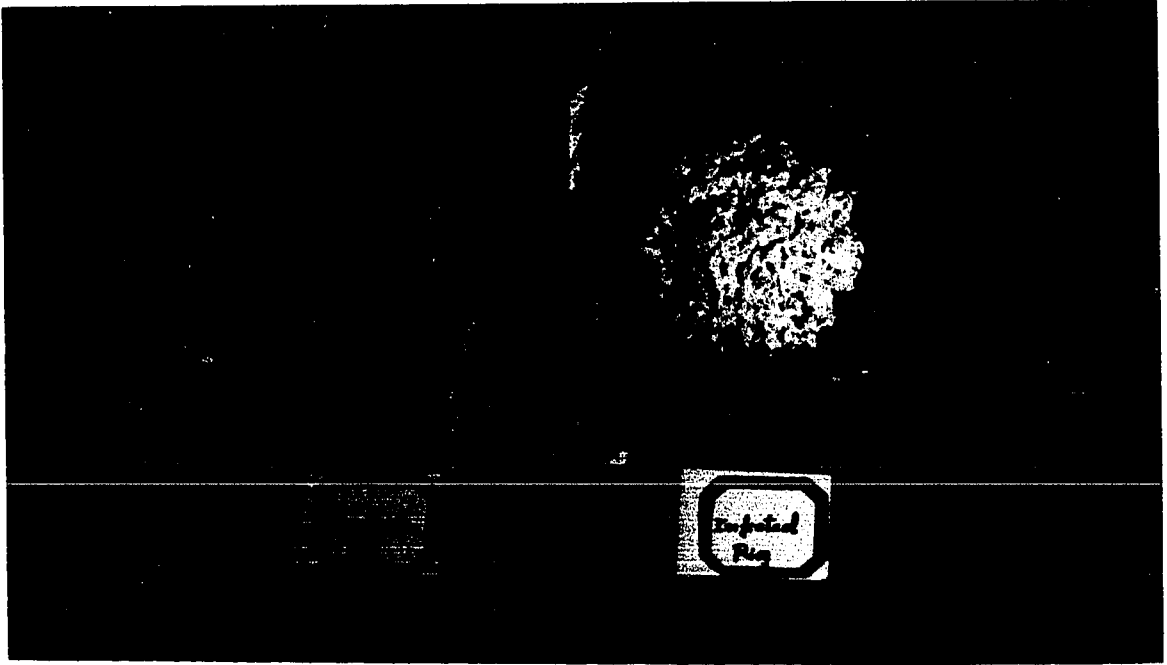
Table 10. The amount of precipitate surfactant, supernatant fraction and total lung lavage materials from one liter of lavage fluid from control and infected pigs

	Sediment surfactant** (mg)	Supernatant fraction** (mg)	Total** (mg)
4th week of infection			
Control (4)[a]	177±19[b]	82±11	259±15
Infected (6)	247±14	243±34	490±45
5th week of infection			
Control (4)	147±12	82±10	228±17
Infected (6)	221±20	298±37	519±54
6th week of infection			
Control (5)	172±9	93±12	266±15
Infected (7)	207±7	297±59	504±62

[a]Number of observations.

[b]Values are means±SEM.

**All differences between control and infected pigs were significant at $P < 0.01$.

The Chemical Composition of Lavaged Materials from the Lungs

The protein composition

The protein content in the sedimented surfactant from the lungs of control pigs varied from 53 to 58 percent, and that from the lungs of the infected pigs varied from 55 to 61 percent. The protein content of the supernatant fraction ranged from 54 to 67 percent in the lavage material from control pigs, while marked increases from 80 to 86 percent were observed in the lavage material from infected pigs. However, the percent of protein in lung tissue itself was 17 percent with no changes following infection (Table 11).

The electrophoretic separation of the proteins in the supernatant fraction showed at least five components. An additional band which migrated ahead of the albumin fraction (reference) was distinguishable as shown in Figures 24, 25 and 26 in the control and experimental group.

The total lipid composition

The lipid content of the sedimented surfactant of the lungs of control pigs varied from 27 to 31 percent while that of the infected pigs varied between 22 and 28 percent (Table 12). The total lipid content of supernatant fraction of the lungs of control pigs showed mean values between 20 and 22 percent. There were significant decreases to 3 and 4 percent (P<0.01) in total lipid in the supernatant fraction from lungs of infected pigs. The total lipid content of lung tissue was fairly constant with mean values ranging between 20 and 22 percent (Table 12).

Table 11.   The protein composition in percent of dry matter of sediment, supernatant, lung tissue

	Sediment %	Supernatant %	Lung tissue %
4th week of infection			
Control (4)[a]	53±3[b]	55±1	16±0.00
Infected (6)	61±2	80±3	17±0.33
5th week of infection			
Control (4)	56±2	67±3	17±0.48
Infected (6)	59±3	86±4	17±0.50
6th week of infection			
Control (5)	58±3	54±3	17±0.37
Infected (7)	55±3	86±2	17±0.31

[a]Number of observations.

[b]Values are mean±SEM.

Fatty Acids Pattern of Sediment Surfactant

There were both saturated and unsaturated fatty acids including myristic acid (14 carbon), palmitic acid (16 carbon), and stearic acid (18 carbon) in the pulmonary sediment surfactant. The fatty acid component present in the greatest concentration was palmitic acid which showed the mean of total percent varied between 70.34 and 76.65 in control pigs (Figures 27, 28, and 29). The rest of the other fatty acids varied between 2.52 and 11.63 percent (Table B2). The percent of saturated fatty

Table 12. The total lipid composition in percent of dry matter of sediment, supernatant, and lung tissue of control and infected pigs

	Sediment surfactant %	Supernatant fraction** %	Lung tissue %
4th week of infection			
Control (4)[a]	30±4[b]	20±3.0	22±1
Infected (6)	22±2	4±0.5	21±1
5th week of infection			
Control (4)	31±3	22±6.0	20±1
Infected (6)	27±3	4±1.0	22±1
6th week of infection			
Control (5)	27±4	22±4.0	20±1
Infected (7)	28±3	3±0.3	20±1

[a]Number of observations.

[b]Values are mean SEM.

**Difference between control and infected pigs was significant at P<0.01.

acids tended to decrease during infection. The percent of unsaturated fatty acids tended to be elevated in the infected pigs (Table B2). The differences, however, were not significant. The mean ratio of total saturated fatty acid:unsaturated fatty acids was 3.79:1, 6.31:1, and 5.19:1 for control pigs at 4th, 5th, 6th week post inoculation. The mean saturated:unsaturated fatty acids ratio decreased to 3.12:1, 4.22:1 and

Figure 24. The electrophoretic separation of protein from 50 ul of supernatant fraction (25 mg/ml) of pigs at 4th week of M. hyopneumoniae infection.

Figure 25. The electrophoretic separation of protein from 50 ul of supernatant fraction (25 mg/ml) of pigs at 5th week of M. hyopneumoniae infection.

Figure 26. The electrophoretic separation of protein from 50 ul of supernatant fraction (25 mg/ml) of pigs at 6th week of M. hyopneumoniae infection.

Control pig   Infected pig   Serum

6th week of infection
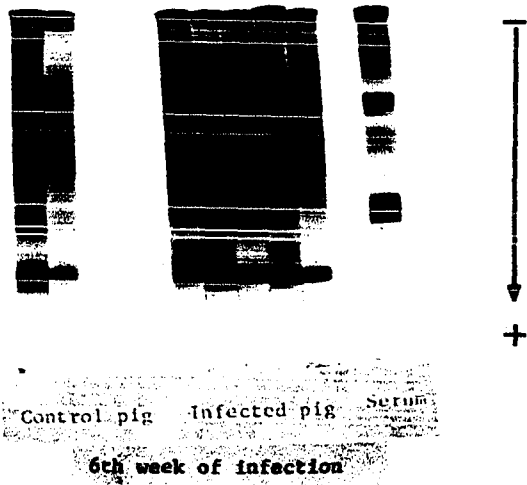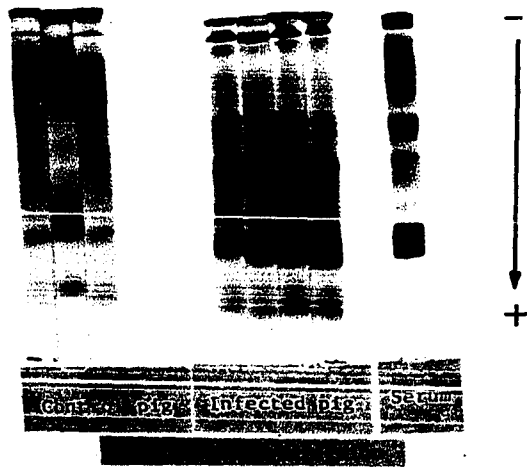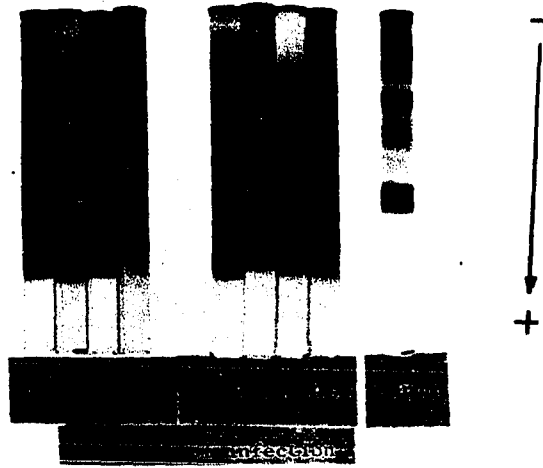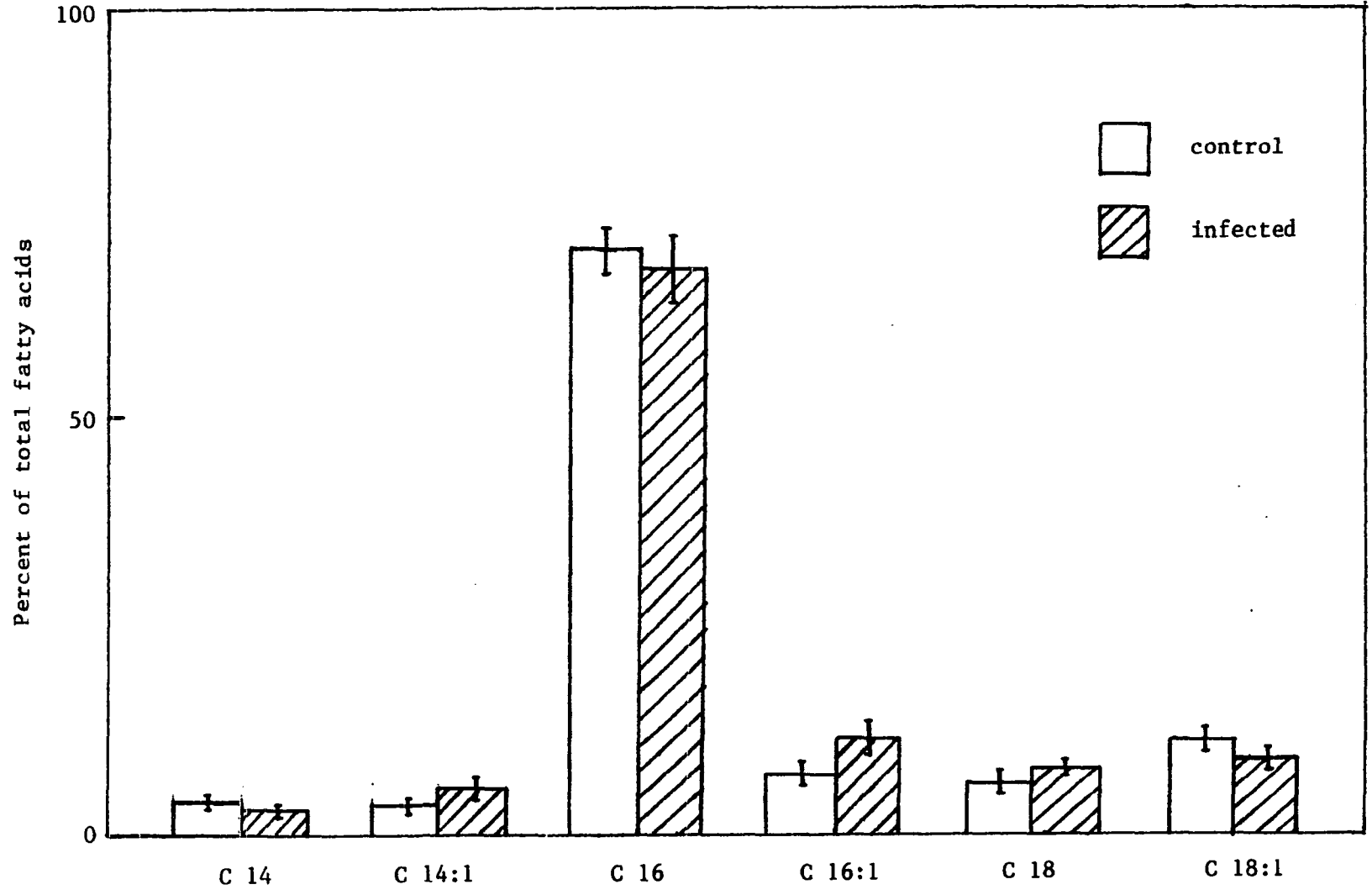
Figure 27. Fatty acid pattern of pulmonary surfactant (sediment) from M. hyopneumoniae infected pigs at 4th week of infection.
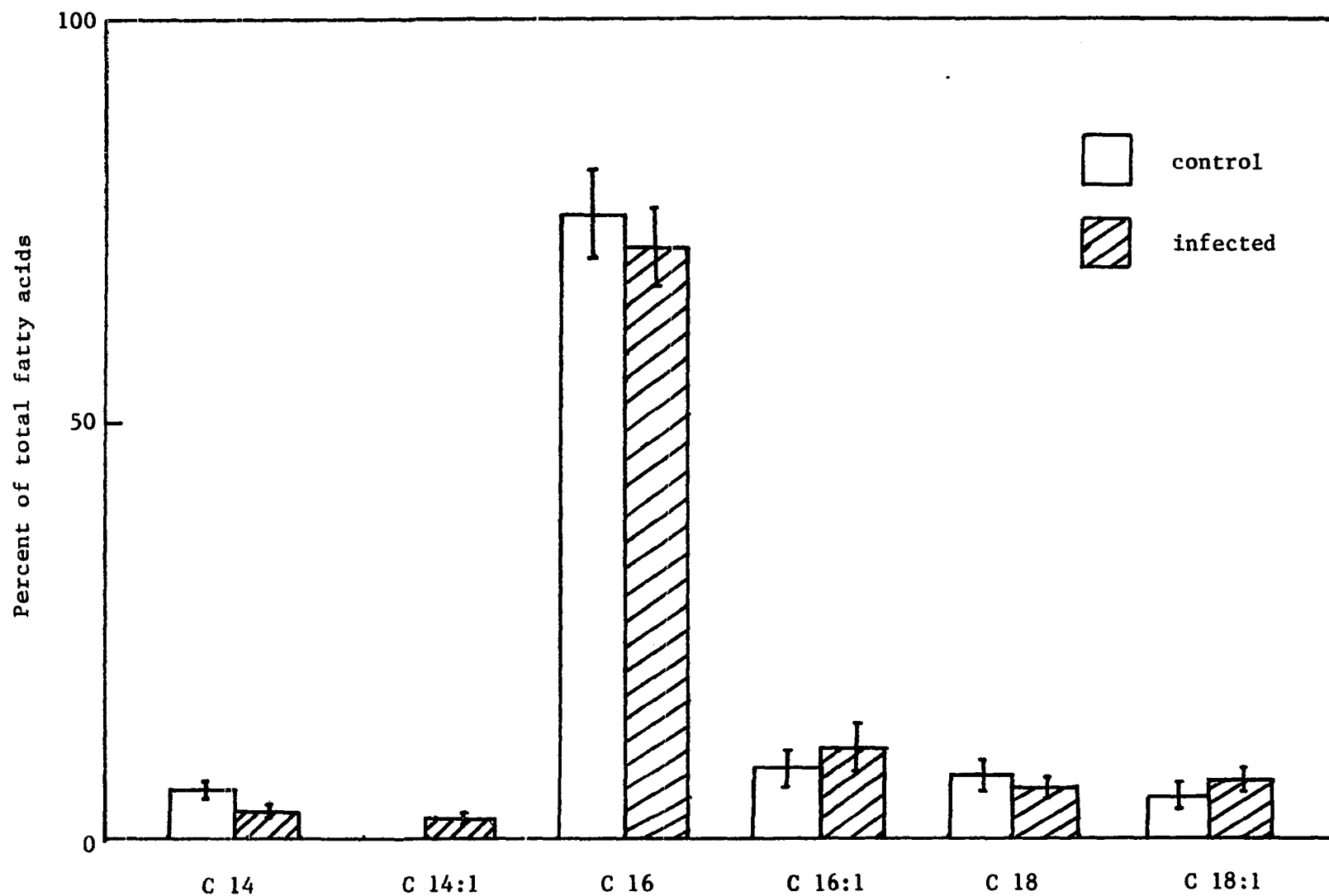
Figure 28. Fatty acid pattern of pulmonary surfactant (sediment) from M. hyopneumoniae infected
pigs at 5th week of infection.
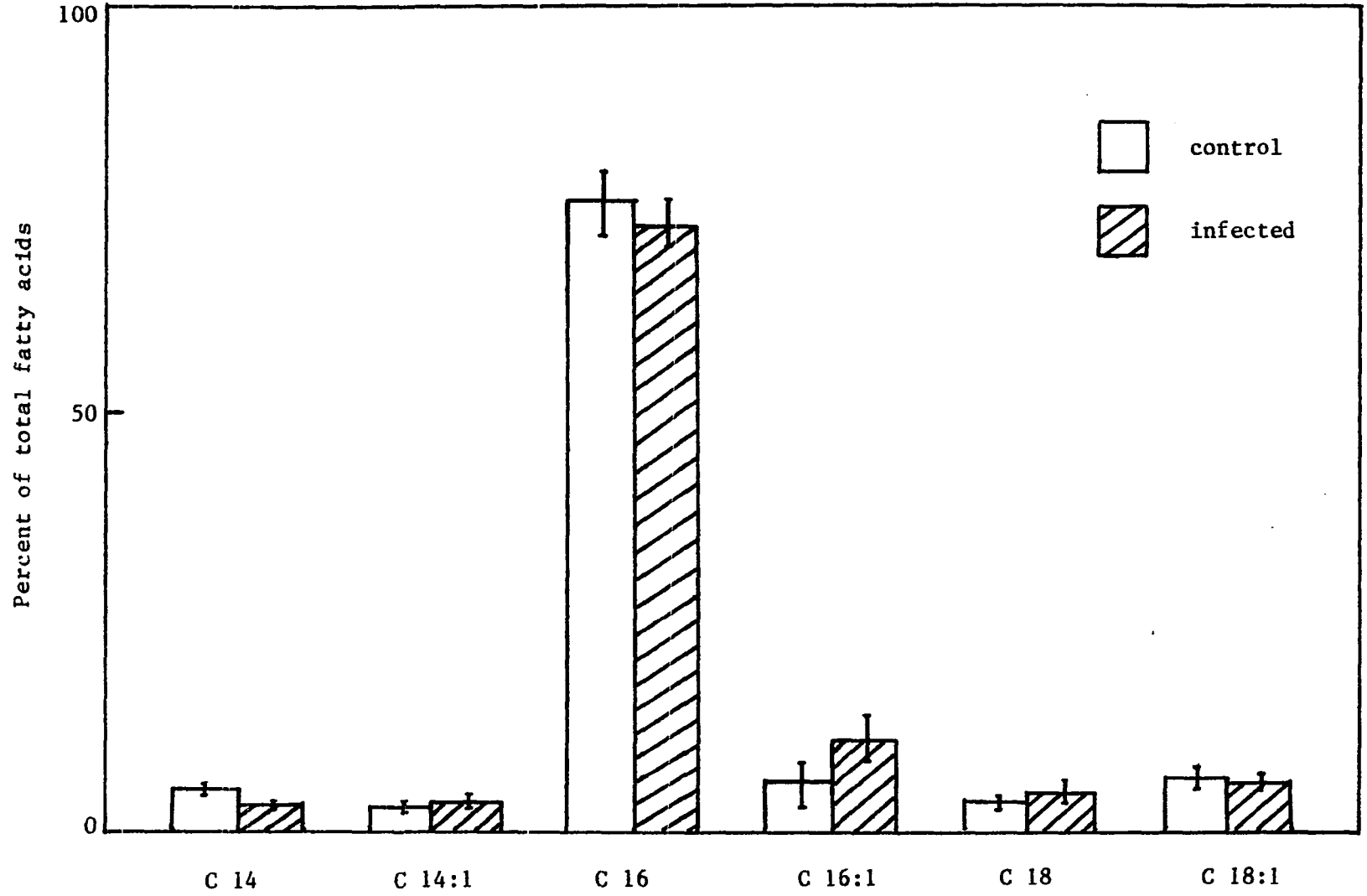
Figure 29. Fatty acid pattern of pulmonary surfactant (sediment) from M. hyopneumoniae infected pigs at 6th week of infection.

4.18:1 for infected pigs at 4th, 5th and 6th week post infection, respectively.

Phospholipids Composition of Sediment
Surfactant and Lung Tissue

The sediment surfactant

The fractionation of phospholipids in the sediment surfactant by thin layer chromatography (Figures 30 and 31) showed that lecithin was the major component, with the mean of percent variation between 79.7 and 81.79 for control pigs. No change in total lecithin occurred with infection as the mean percent for these pigs varied from 78.39 to 81.19. The concentration of ethanolamine varied between 7.88 and 9.44 percent, and that of sphingomyelin and lysolecithin varied between 10.33 and 12.35 percent. The relative amount of ethanolamine and sphingomyelin and lysolecithin in the infected pigs was not different from that of the control animals (Figure 32, Table 13 and Table B3). In the control pigs the means of the total phospholipid concentration of sediment were between 232.00 and 261.25 mg per gm of dry matter. Marked decreased which varied between 201.50 and 222.86 mg per gm of dry matter occurred in infected pigs.

The lung tissue

Lecithin was the major component of the lung tissue. The means of the percent concentration varied between 55.44 and 55.63, with slight decreases in the infected pigs. The mean of percent of ethanolamine varied between 17.23 and 18.01 and was changed very little in the infected pigs. Shpingomyelin and lysolecithin had mean values which varied between 24.06

Figure 30. The fractionation of phospholipids from 500 ugm of total
lipid from surfactant on thin layer chromatography plate.
The solvent system consisted of chloroform:methanol:
water; 80:25:4.

Figure 31. The fractionation of phospholipids from 500 ugm of total
lipid from lung tissue on thin layer chromatography plate.
The solvent system consisted of chloroform:methanol:water;
80:25:4.

Solvent Front
Chol.

Ethano.

Lecithin.

SPingo.

LYso.

Origin

Solvent Front
Chol.

Ethano.

Lecithin.

Sphingo.

Lyso.

Origin

Control   Infected   Std.
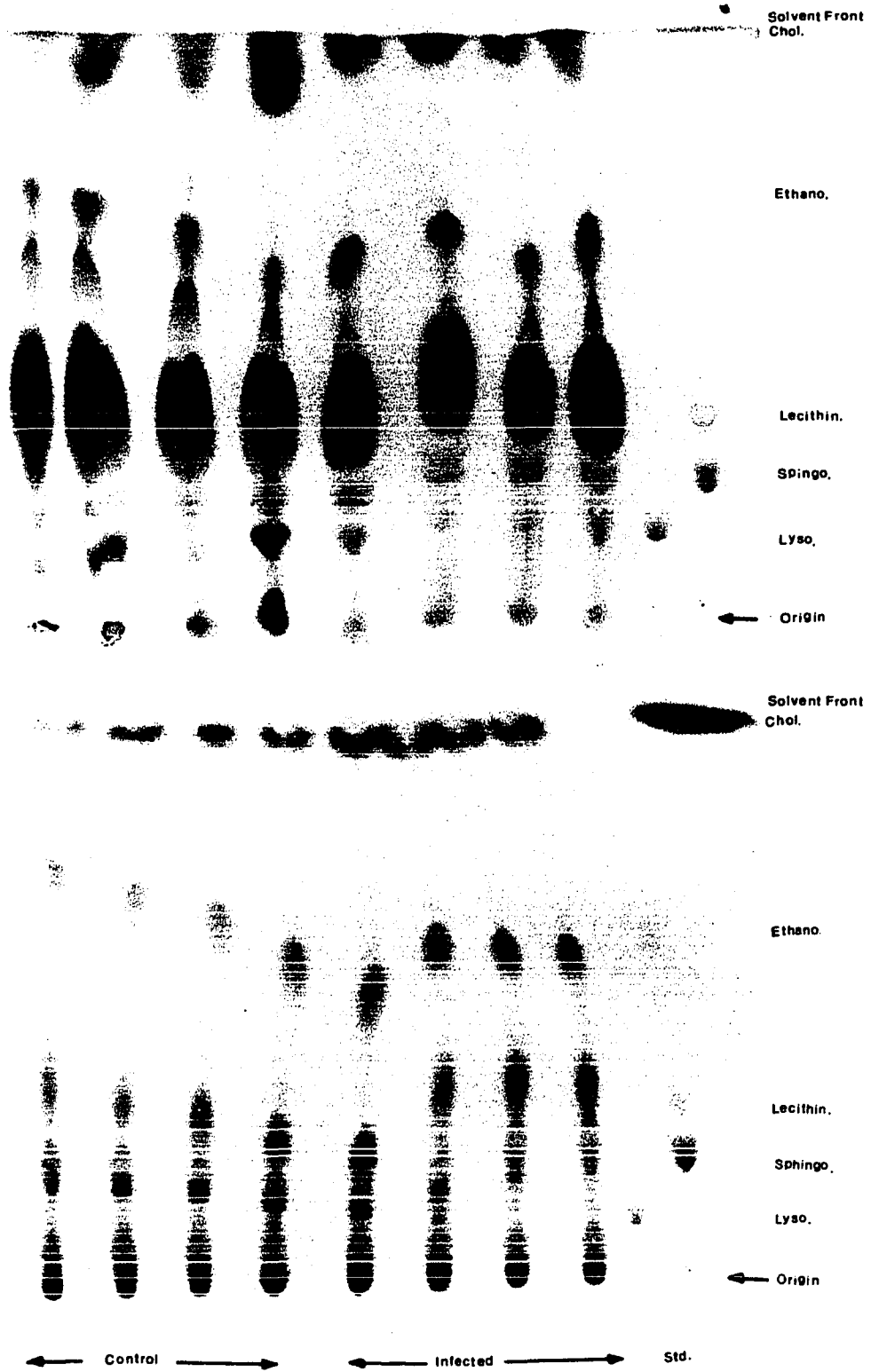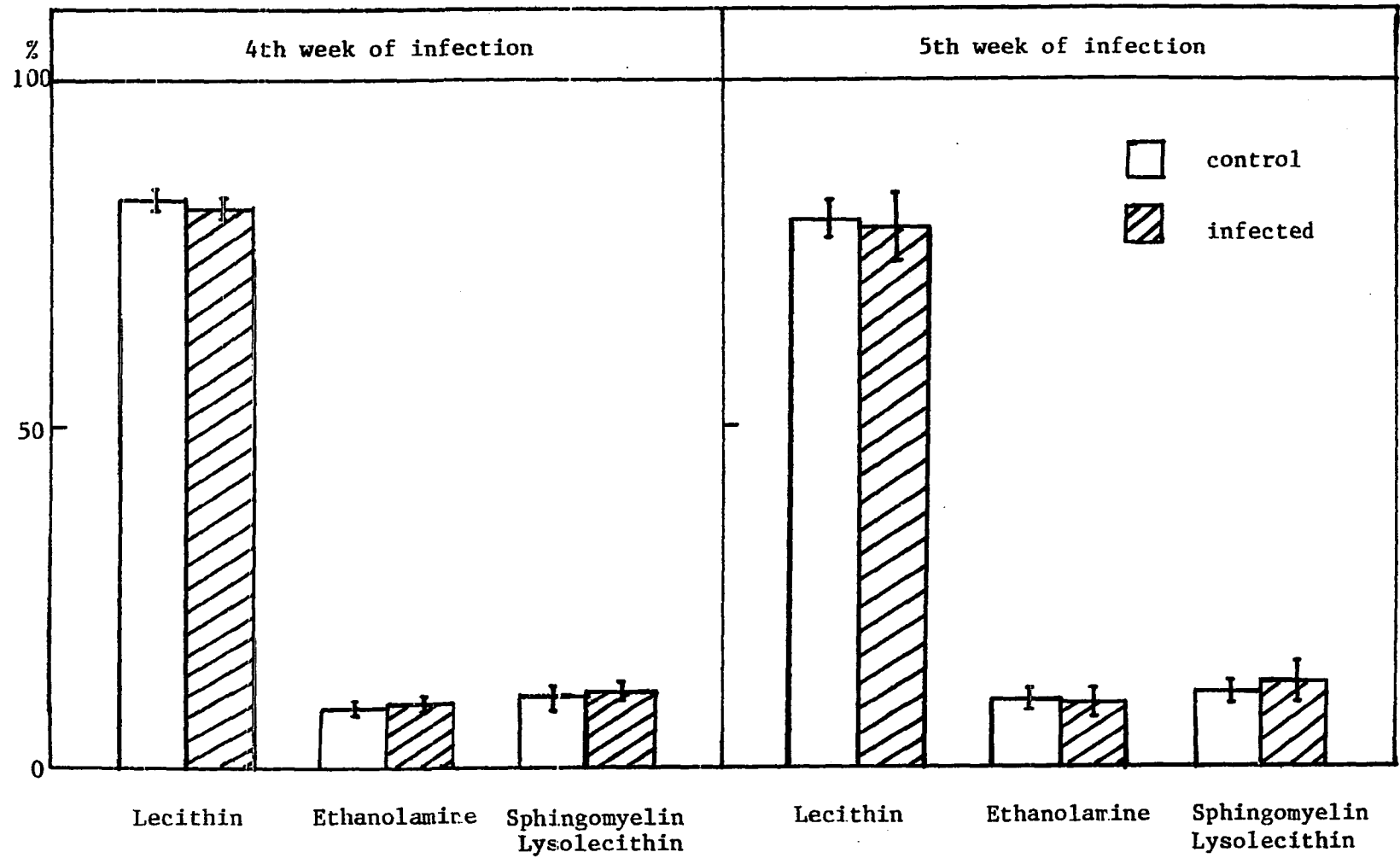
Figure 32. The percent composition of phospholipid fractions in the pulmonary surfactant (sediment).

Table 13. Phospholipid concentration of sediment surfactant and lung
tissue of control and infected pigs

	Sediment surfactant[a] (mg/gm DM)	Lung tissue (mg/gm DM)
4th week of infection		
Control (4)[b]	261.25±19.16[c]	158.25±5.31
Infected (6)	201.50±14.08	143.17±4.39
5th week of infection		
Control (4)	232.00±24.33	154.00±6.22
Infected (6)	214.00±15.95	158.17±8.01
6th week of infection		
Control (5)	251.80±10.58	166.20±9.52
Infected (7)	222.86±15.66	145.57±3.98

[a]Phospholipid concentration was calculated from the concentration of
P x 22.88.

[b]Number of observations.

[c]Values are mean±SEM.

and 27.15 and were only slightly changed in infected pigs (Table B3). The
mean total phospholipid concentration in the lung tissue was between
150.00 and 166.20 mg per gm and tended to decrease slightly in infected
pigs. The mean concentrations in lung tissue of infected pigs varied
between 143.17 and 158.17 mg per gm dry matter (Figure 33, Table 13 and
Table B3).
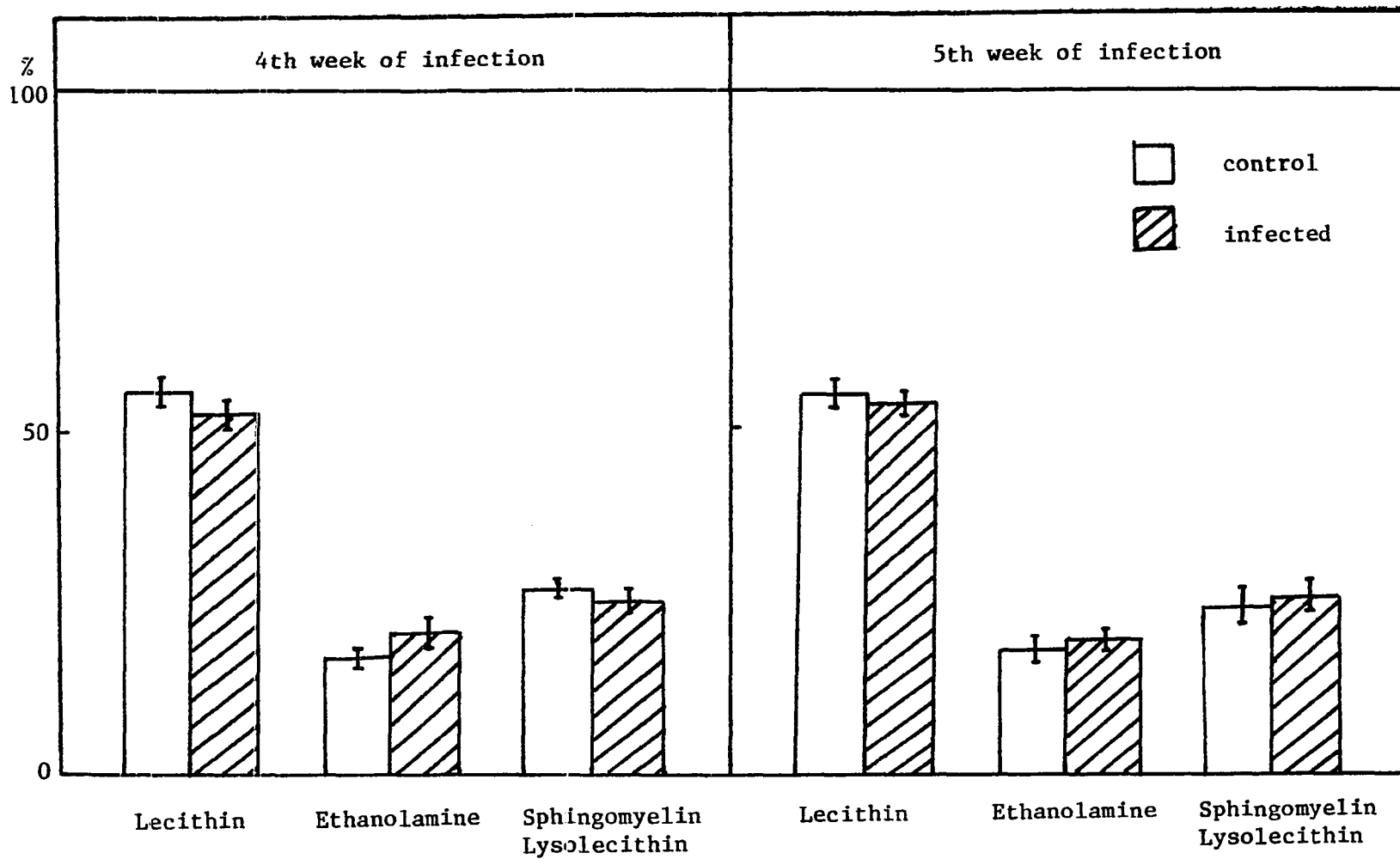
Figure 33. The percent composition of phospholipid fractions in the lung.

The Distribution of Palmitate-^{14}C in Sediment

Surfactant, Lung Tissue and Plasma

The sedimented surfactant, lung tissue, and plasma were collected at five hours after intravenous injection of palmitate-^{14}C. The mean of radioactivity of sedimented surfactant was between 1913 to 2053 DPM per 20 mg of dry matter for control pigs. The mean values for infected pigs decreased to between 1610 and 1821 DPM per 20 mg dry matter.

The mean radioactivity of the lung tissue in control pigs ranged from 783 to 916 DPM per 20 mg dry matter. There was a decrease ($P<0.01$) in the radioactivity for the infected pigs which showed the mean values ranging from 573 to 590 DPM per 20 mg dry matter. The mean radioactivity of the plasma ranged from 342 to 350 DPM per ml for both the control animals and infected animals (Table 14).

The Distribution of Radioactivity of Palmitate-^{14}C

Between the Phospholipid Fractions in Pulmonary

Surfactant (Sediment) and Lung Tissue

The means of the percent of radioactivity in lecithin component of the sediment were between 89.88 and 90.34 for the control pigs. The radioactivity decreased slightly in the infected pigs. The means of percent of radioactivity in ethanolamine component were between 4.10 and 4.15 for control animals, and also showed slight decreases in the infected animals. The means of percent of radioactivity distribution in the sphingomyelin and lysolecithin components of control animals were between 5.50 and 6.04.

Table 14. Distribution of palmitate-^{14}C in sediment, lung tissue and plasma in DPM

	Sediment (20 mg)	Lung tissue** (20 mg)	Plasma (1 ml)
4th week of infection			
Control (4)[a]	1913±195[b]	783±69	346±18
Infected (4)	1821±244	573±33	350±43
5th week of infection			
Control (4)	2053±78	916±27	342±7
Infected (4)	1610±215	590±61	343±22

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at $P<0.01$.

There were slight increases in radioactivity in the infected pigs (Figure 34 and Table B4).

The mean of percent of radioactivity in the lecithin component of lung tissue was between 64.25 and 72.34 in control pigs and 64.06 and 71.77 for infected pigs. The ethanolamine component had means which varied between 10.39 and 11.72 for control pigs. The radioactivity distribution showed marked decrease for the lecithin and ethanolamine components in both sediment and lung tissue. The sphingomyelin and lyso-lecithin component showed the means varied from 5.50 to 6.04 and in the lung tissue were between 17.29 and 24.04 in the control animals. There
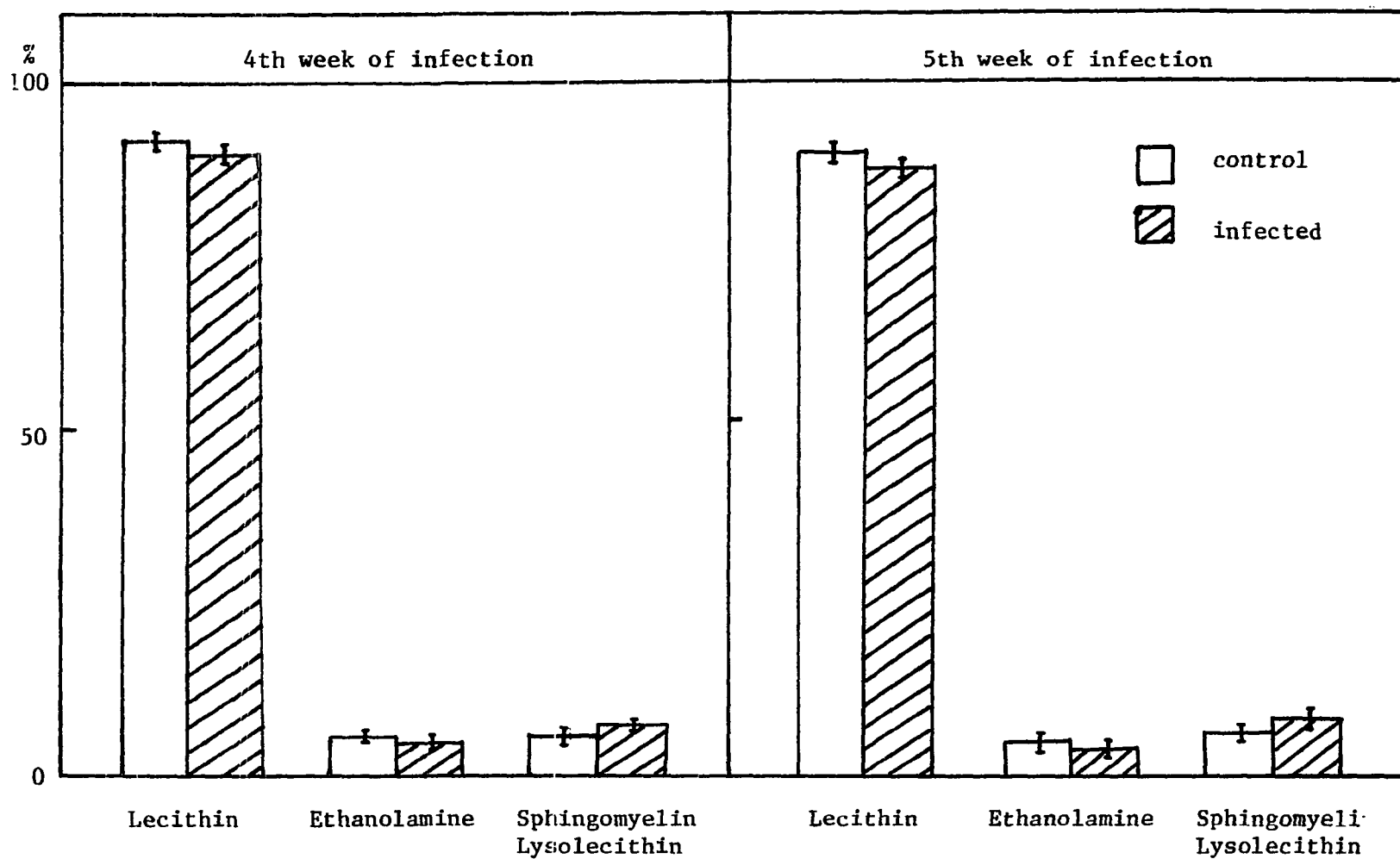
Figure 34. Distribution of radioactivity between phospholipid fractions in pulmonary surfactant (sediment) 5 hours following the intravenous administration of ^{14}C labeled palmitate.

were slight increases of the radioactivity for the infected animals
(Figure 35 and Table B4).

<div align="center">

The Total Concentration of Sialic Acid in Pulmonary

Sediment Surfactant, Supernatant Fraction,

Plasma and Lung Tissue

</div>

The total amount of sialic acid was determined for the sedimented
surfactant and the supernatant fraction obtained from one liter of lavage
fluid. The mean values for sialic acid in dry matter of sedimented sur-
factant were between 11.37 and 17.19 mg for control pigs. Marked in-
creases were observed in the lavage material from infected pigs which
showed the mean values ranging from 18.15 to 20.59 mg. The total amount
of sialic acid in dry matter of supernatant fraction of lavage fluid from
control pigs varied between 6.76 and 15.06 mg. There were significant
increases ($P<0.01$) for sialic acid for lavage supernatant for the infected
pigs. These mean values were between 41.85 and 46.64 mg.

The sialic acid concentration in the plasma showed the means varied
from 64.69 to 66.97 mg per 100 ml of plasma in control pigs. The infected
pigs had mean sialic acid concentrations ranging between 65.14 and 73.18
mg per 100 ml of plasma, with increases paralleling the duration of infec-
tion.

The mean concentration of sialic acid in the lung tissue of both
groups varied from 4.26 to 4.88 mg per gm of dry matter (Table 15).
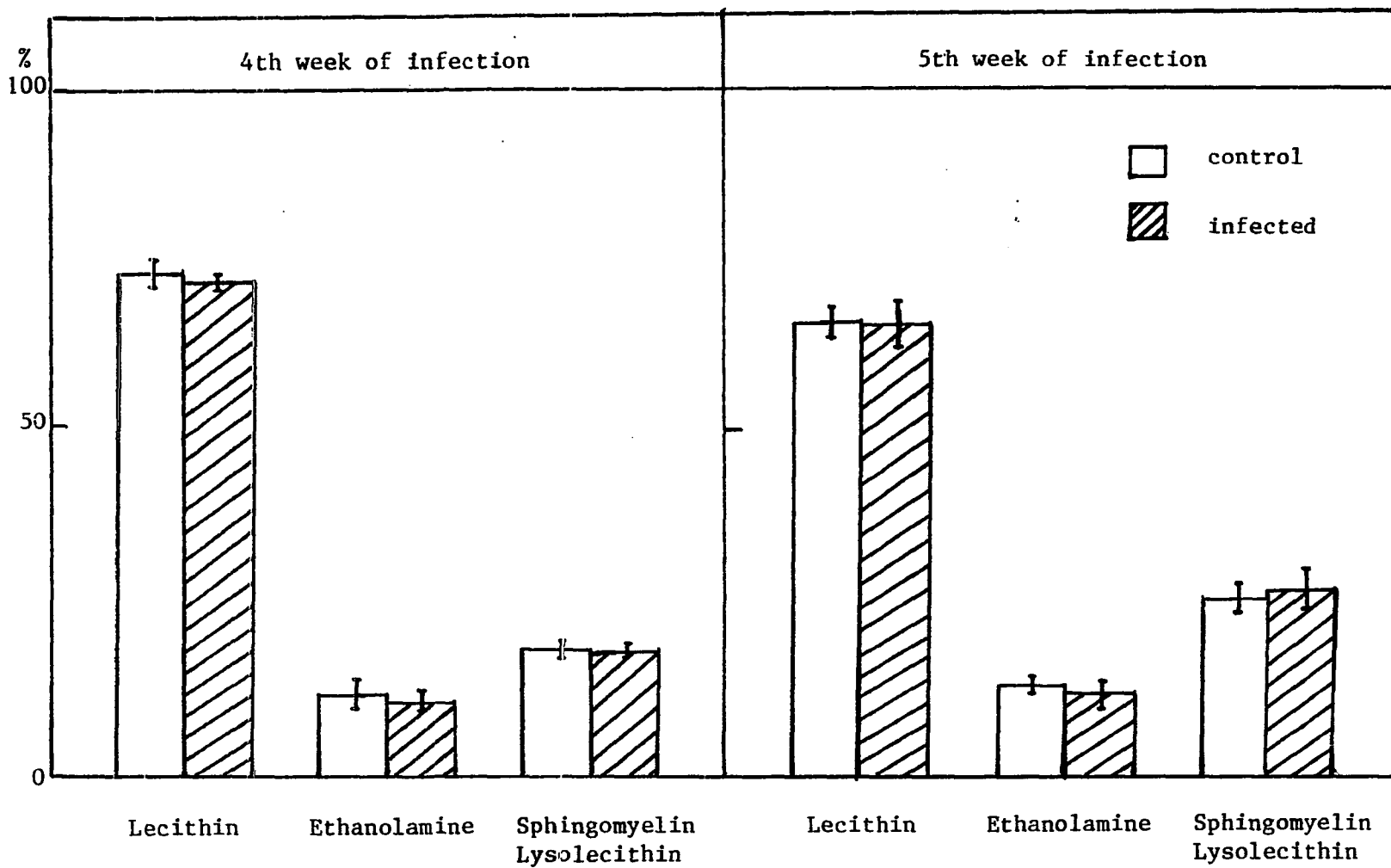
Figure 35. Distribution of radioactivity between phospholipid fractions in lung 5 hours following the intravenous administration of ^{14}C labeled palmitate.

Table 15. Sialic acid concentration in sediment, supernatant, total pulmonary surfactant, plasma, and lung tissue

	Sediment (mg)	Supernatant** (mg)	Total** (mg)	Plasma (mg %)	Lung tissue (mg/gm DM)
4th week of infection					
Control (4)[a]	11.37±0.48[b]	6.76±1.33	18.13±1.73	64.69±5.11	4.26±0.12
Infected (6)	18.15±1.37	46.64±11.23	64.80±11.96	65.14±2.14	4.43±0.13
5th week of infection					
Control (4)	12.66±1.29	13.80±2.15	26.46±1.83	65.86±2.60	4.42±0.11
Infected (6)	20.59±2.06	51.98±6.85	72.57±8.44	69.00±4.28	4.48±0.07
6th week of infection					
Control (5)	17.19±1.26	15.06±2.25	32.25±3.09	66.97±1.11	4.88±0.28
Infected (7)	18.48±1.69	41.85±7.88	60.33±8.39	73.18±2.10	4.61±0.21

[a]Number of observations.

[b]Values are mean±SEM.

**Difference between control and infected pigs was significant at P<0.01.

DISCUSSION

Mycoplasma hyopneumoniae has been named and characterized as the causative agent for a chronic pneumonia of swine (Maré and Switzer, 1965). The disease is distributed worldwide and has caused an enormous economic loss to the swine industry as emphasized by Switzer and Ross (1975). The clinical signs of mycoplasmal pneumonia appear after an incubation period of 10-16 days and include coughing, loss of vigor, growth retardation, decreased efficiency of feed utilization, high morbidity and low mortality. These effects will be severe if there are secondary bacterial infections. The pathologic lesions of the lung have been well-described (Lam, 1970; Livingston et al., 1972; Oboegbulem, 1973). Most of the gross lesions occur in the infected areas of the lung resulting in atelectasis which is followed by consolidation. The most extensive damages of the lung occurred at about 28 days after intranasal inoculation (Livingston et al., 1972). Switzer and Preston (1974) found that young growing pigs were most susceptible to infection.

In this study young pigs were infected at 5 1/2 to 6 weeks of age and physiological studies were made. The growth rate of infected pigs was compared with that of the control pigs. The body weight gain of infected pigs decreased by 9.50, 7.31 and 14.67 percent at 4th, 5th, and 6th week of infection, respectively. The reduction of growth rate in mycoplasmal pneumonia group of swine was quite obvious in the young growing pigs.

Coughing was observed as early as two weeks post inoculation. The characteristic pattern of breathing was normal and there was not respiratory discharge. This finding was similar to previous descriptions (Betts,

1952; L'Ecuyer and Switzer, 1963; Oboegbulem, 1973). There was no significant difference in body temperature between infected and control pigs. There was no significant difference in either heart rate or respiratory rate between control and infected animals.

Most of the gross lesions in the pneumonic lungs occurred in the apical, cardiac and intermediate lobes and appeared as consolidated areas which were well-demarcated and plum-red in color. Lesions occurred in the upper region of diaphragmatic lobe in many infected animals. The weight of pneumonic lungs per kg body weight was increased markedly and the lungs were edematous. The gross changes were similar in the pneumonic lungs of pigs at the 4th, 5th, and 6th week of infection.

The microscopic lesions of the lung of infected pigs were similar to those described in many previous reports (Lam, 1970; Livingston et al., 1972; Oboegbulem, 1973). There was hyperplasia of the peribronchial and perivascular cells. Leukocytes invaded the alveoli and then the lumen of bronchioles. The peribronchiolar lymphatic nodules were hypertrophied. There was a tremendous amount of exudate and secretion from the respiratory tract of the infected pigs. Cartarrha from peribronchiolar cells also was observed. It was postulated from this experiment that M. hyopneumoniae caused damage to the lung structure producing atelectasis and eventually consolidation. The consolidated areas were dense with neither gas movement nor perfusion and were scattered in the apical, cardiac, intermediate and the upper part of diaphragmatic lobes. The infected lung produced a great amount of secretion as can be seen by lung lavage fractions. The thickening of vascular membranes and stiffness of bronchi-

oles could contribute to the change of the respiratory parameter values. The results of respiratory measurements from the experiment showed the following changes:

1. The infected pigs consumed significantly less oxygen, even with significant increases in tidal volume. The reason for this is the capability of gas diffusion was decreased.

2. There were drastic increases in the expiratory and inspiratory flow rate of air.

3. The minute volume and alveolar ventilation were increased significantly in the infected animals. Forster (1974) pointed out that hyperventilation is a response to an increase in body fluid acidity.

The transpulmonary pressure was increased significantly in infected pigs. The dynamic lung compliance decreased markedly in infected pigs. This means that elasticity of the lung was reduced and, also, that the airway resistance increased. The hypersecretion of nonsurfactant substances into the lung occluded the airway causing these changes in compliance and resistance. The work of breathing subsequently increased (P<0.01) in infected pigs attempting to overcome this problem. It was assumed that the infected pigs would spend more energy for respiratory compensation. As a result of M. hyopneumoniae infection, the affected alveolar areas collapsed (atelectasis), and the invasion of leukocytes, exudates and cellular debris into alveolar pores occluded the bronchioles and airways. This reduced the areas available for gas exchanges. The infected animals showed marked increases in total lung weight and evidence of edema.

The edematous development would reduce the rate of blood flow in the capillary system. As a result of a poor diffusion of oxygen at the alveoli there was a marked decrease in partial pressure of oxygen in arterial blood. Whenever gas diffusion is decreased, the pH of arterial blood will decrease. However, in the present studies there were slight decreases in the pH of arterial blood and in the partial pressure of arterial blood PCO_2 to values less than those of the control animals. The reason for this is that the buffer system of the blood operated to neutralize the excess H^+ as indicated by marked decrease in the HCO_3^-, and significant decreases of base excess in infected animals. The other evidence of hyperventilation was significant decrease in alveolar PCO_2 in infected animals. Infected animals attempted to decrease the $PACO_2$ level by hyperventilation, with the result that the alveolar PCO_2 decreased blood pH values in most infected pigs remained near normal because of compensatory respiratory alkalosis (hyperventilation). West (1974) pointed out that a balance $\dot{V}/\dot{Q}$ ratios do exist in normal lung. However, if the perfusion is decreased, the enhancement of diffusion of gas must be increased so as to maintain the balance of acid-base and gas concentration of blood. Therefore, it can be assumed that in the mycoplasmal pneumonic lung greater ventilation is required because of the reduction of pulmonary circulation. Pigs infected with M. hyopneumoniae could show possibly both poor diffusion of gas and poor circulation in the capillaries of the lung.

There were no drastic changes in hematologic components in pigs with mycoplasmal pneumonia. No significant differences were observed between the infected and control animals in hemoglobin, packed cell volume, or

number of red blood cells. Serum electrolytes such as sodium, potassium, and chloride remained at a normal level. There were marked increases in number of white blood cells in infected animals. The leukocytes increased to between 18.40 x 10^3 and 19.81 x 10^3 cells/cmm. The most consistent leukocyte alteration in pigs with mycoplasmal pneumonia was a marked increase of neutrophils and marked decrease of lymphocytes. The number of neutrophils increased in parallel with the increase in total white blood cells. Monocytes, eosinophils, and basophils also showed slight increases. There were transient reductions in lymphocyte counts.

Mean total plasma protein concentrations in normal pigs ranged from 5.25 to 5.65 gm/100 ml and in infected pigs from 6.33 to 6.53 gm/100 ml. Electrophoretic separation of the various fractions indicated a reduction in albumin and an increase in globulins. The increase in relative amounts of globulin resulted primarily from an elevation of alpha-2 globulin. However, this type of change is common in many diseases and even some kinds of stress could induce this result (Vesselinovitch, 1955). Hematologic changes were for the most part generalized reactions to inflammation. Results of respiratory parameters and blood gas analysis offered more information concerning the acid-base balance status in mycoplasmal pneumonia pigs.

Significant increases in amount of sediment surfactant and supernatant fraction were observed in infected animals. Phospholipids are the major component of pulmonary surfactant and play a major role in stabilization of the alveolar surface tension (Clements et al., 1958; Tierney et al., 1967; Scarpelli, 1968a; Clements, 1970; King, 1974). The results from the present experiment showed that lecithin was the major

component of phospholipids. Ethanolamine, sphingomyelin and lysolecithin were incorporated as minor components of total phospholipids. There was a slight decrease in the lecithin concentration of sediment surfactant in infected pigs as compared with that of normal pigs. However, the total amount of sediment surfactant in lavage fluid of the infected pigs showed a significant increase. This result could be due to an increased synthesis of phospholipid to maintain the normal patent alveoli.

Cohen and Somerson (1967) showed that Mycoplasma pneumoniae can produce hydrogen peroxide which is harmful to the host cells. The ratio of saturated fatty acid:unsaturated fatty acid in sediment surfactant decreased in the M. hyopneumoniae infected pigs. A slight reduction of saturated fatty acids and a slight elevation in percent of unsaturated fatty acids occurred. By injecting palmitate-^{14}C into pigs with mycoplasmal pneumonia, it was found that there was a slight decrease of radioactivity in sediment surfactant but a significant decrease of radioactivity in the lung tissue. The net amount of radioactivity in sediment surfactant from one liter of lavage fluid from infected animals was greater than that from the control group. This indicated an increased synthesis of phospholipids in infected pigs.

Gesner and Thomas (1965) reported that sialic acid was the binding site of M. gallisepticum to erythrocytes and this carbohydrate plays a role in hemagglutination. Sobeslavsky et al. (1968) showed that sialic acid component of tracheal epithelial cells or blood cells was the adsorption site for M. pneumoniae. Although the sialic acid content of sediment surfactant showed only a slight increase, there was significant

increase in total amount of sialic acid in surpernatant fraction of the lung lavage of infected pigs. An electrophoretic analysis of plasma glycoprotein revealed a marked increase in glycoprotein in infected pigs. From the results of the determination of concentration of sialic acid and glycoprotein in the pulmonary secretion and plasma of infected pigs, it would be postulated that the infected lung secreted glycoprotein containing sialic acid to prevent the attachment of Mycoplasma hyopneumoniae or reduce the proliferation of the number of pathogenic agents.

SUMMARY

The primary concern of this research was to determine the physiologic changes in the lung of young growing pigs that were intratracheally inoculated with Mycoplasma hyopneumoniae. Hematologic changes were also studied. All studies were performed at the 4th, 5th and 6th week post inoculation of 5 1/2 to 6 week old pigs.

The infected pigs showed marked decreases of body weight gain. There were no significant changes of heart rate, respiratory rate, and body temperature. At autopsy the apical, cardiac, intermediate and the upper part of diaphragmatic lobes of the lungs of infected pigs showed a scattering of well-demarcated pneumonic lesions. The lungs of infected pigs increased in weight and developed edema.

There were several changes in respiratory parameters in infected animals. The infected pigs showed a significant increase in esophageal pressure negativity, expiratory flow rate, tidal and minute volume, alveolar ventilation and work of breathing. There was significantly less oxygen consumption. There were marked increases of inspiratory flow rate and airway resistance in infected pigs. The elasticity of infected lungs was reduced markedly as shown by marked decreases of dynamic lung compliance.

Blood gas analyses showed that there were changes in acid-base balance in infected pigs. The pH of arterial blood in infected animals was decreased and the arterial partial pressure of O_2, and CO_2 and arterial concentration of HCO_3^- showed marked decreases. The blood of infected pigs showed some degree of respiratory acidosis as indicated by

significant decreases of base excess. As a result the infected animals operated compensatory respiratory alkalosis by increased hyperventilation in order to maintain the pH of arterial blood near normal.

The pigs with mycoplasmal pneumonia showed no drastic hematologic changes. There were no changes in red blood cell number, packed cell volume, or hemoglobin. However, the total number of white blood cells increased markedly in infected pigs. The differential count revealed large increases in the number of neutrophils, small increases in number of monocytes, eosinophils and basophils, and slight decreases in numbers of lymphocytes. The total plasma protein and glycoprotein concentrations were increased significantly in infected animals. The electrophoretic separation of plasma proteins showed a significant decrease in relative percent of albumin and significant increase in relative percent of globulin fractions. Plasma glycoprotein increased markedly in infected pigs as well. There were no significant changes in concentrations of serum electrolyte such as $Na+$, $K+$, and Cl^-. Blood glucose and cholesterol showed no changes.

Special emphasis was placed on determining the changes in the lung surfactant system. The total amount of sediment surfactant that was collected by lung lavage increased significantly in the infected animals. The fractionation of phospholipids of sedimented surfactant on thin-layer chromatography indicated there were no significant differences in the individual phospholipids between control and infected lung. When phospholipids were labeled with palmitate-^{14}C there was slightly less radioactivity incorporated into the sedimented surfactant, and significantly less radioactivity incorporated into lung tissue of infected pigs.

131

However, there were no significant differences between control and infected animals in the amount of radioactivity in the plasma.

The chemical analyses of dried lung lavage materials showed that the total percent of protein and lipid of sedimented surfactant were not significantly different between control and infected pigs. There were significant increases in protein concentration and decreases in total percent lipid in the supernatant fraction of lavage substances from the lungs of infected animals.

There were significant increases in total concentration of sialic acid in the sedimented surfactant and supernatant fraction of the lavage. There was also a marked increase of sialic acid concentration in plasma of infected pigs.

REFERENCES

Abrams, M. E. 1966. Isolation and quantitative estimation of pulmonary surface active lipoprotein. J. Appl. Physiol. 21: 718-720.

Adams, F. H. and T. Fujiwara. 1963. Surfactant in fetal lamb tracheal fluid. J. Pediat. 63: 537-542.

Adams-Mayne, M. and D. N. Ward. 1964. Stability studies in luteinizing hormone. Endocrinology 75: 333-340.

Agostoni, E., A. Taglietti and I. Setnikar. 1957. Absorption force of the capillaries of the visceral pleura in determination of the intrapleural pressure. Am. J. Physiol. 191: 277-282.

Ambrose, E. J. and F. J. C. Roe. 1966. The biology of cancer. Van Nostrand, Princeton, New Jersey.

Amir, S. M., S. A. Barker, W. R. Butt and A. C. Crooke. 1966. Purification and lability of human follicle-stimulating hormone. Nature (London) 209: 1092-1093.

Anthonisen, N. R., J. Danson, P. C. Robertson and W. R. D. Ross. 1969. Airway closure as a function of age. Resp. Physiol. 8: 58-65.

Apthorp, G. H. and R. Marshall. 1961. Pulmonary diffusing capacity: A comparison of breath-holding and steady state methods using carbon monoxide. J. Clin. Invest. 40: 1775-1784.

Askin, F. B. and C. Kuhn. 1971. The cellular origin of pulmonary surfactant. Lab. Invest. 25: 260-268.

Avery, M. E. and J. Mead. 1959. Surface properties in relation to atelectasis and hyaline membrane disease. Am. J. Dis. Child. 97: 517-523.

Ballard, P. L. and R. A. Ballard. 1972. Glucocorticoid receptors and the role of glucocorticoids in fetal lung development. Proc. Nat. Acad. Sci. USA 69: 2668-2672.

Bates, D. V., P. T. Macklem and R. V. Christie. 1971. Respiratory function in disease. Second edition. W. B. Saunders Co., Philadelphia.

Bedell, G. N. and R. W. Adams. 1962. Pulmonary diffusing capacity during rest and exercise. A study of normal persons and persons with atrial septal defect, pregnancy, and pulmonary disease. J. Clin. Invest. 41: 1908-1914.

Bensch, K., K. Schaefer and M. E. Avery. 1964. Granular pneumonocyte electron microscopic evidence of their exocrinic function. Science 145: 1318-1319.

Betts, A. O. 1952. Respiratory disease of pigs. V. Some clinical and epidemiological aspects of virus pneumonia of pigs. Vet. Rec. 64: 283-288.

Bhattacharyya, S. N., M. A. Passero, R. P. DiAugustine and W. S. Lynn. 1975. Isolation and characterization of two hydroxyproline-containing glycoproteins from normal animal lung lavage and lamellar bodies. J. Clin. Invest. 55: 914-919.

Bhattacharyya, S. N., M. C. Rose, M. G. Lynn, C. MacLeod, M. Alberts and W. S. Lynn. 1976. Isolation and characterization of a unique glycoprotein from lavage of chicken lungs and lamellar organelles. Am. Rev. Res. Dis. 114: 843-850.

Blumenfeld, T. A., J. M. Driscoll, Jr. and L. S. James. 1974. Lecithin/spingomyelin ratio in tracheal and pleurygeal aspirates in RDS. J. Pediat. 85: 403-407.

Body, D. R. 1971. The phospholipid composition of pig lung surfactant. Lipids 6: 625-629.

Bogden, A. E. and J. H. Gray. 1968. Glycoprotein synthesis and steroids. II. α-2-GP synthesis another parameter for the study of glucocorticoids. Endocrinology 82: 1085-1091.

Bogden, A. E., E. M. Glenn, T. Koslowski and C. S. Rigiero. 1967. α-2-glycoprotein response in adjuvant-induced polyarthritis: An immunoassay for anti-inflammatory activity. Life Sci. 6: 965-973.

Bogden, A. E., J. H. Gray and C. S. Rigiero. 1968. Glycoprotein synthesis and steroids. III. An immunoassay for the anti-inflammatory activity glucocorticoids. Endocrinology 82: 1093-1097.

Boulanger, P. and C. L'Ecuyer. 1968. Enzootic pneumonia of pigs: Complement fixation tests for the detection of mycoplasma antibodies in the serum of immunized rabbits and infected swine. Can. J. Comp. Med. 32: 547-554.

Brumley, G. W., V. Chernick, W. A. Hodson, C. Normand, A. Fenner and M. E. Avery. 1967. Correlations of mechanical stability, morphology, pulmonary surfactant, and phospholipid content in the developing lamb lung. J. Clin. Invest. 46: 863-873.

Bryan, A. C., J. Milic-Emili and D. Pengelly. 1966. Effect of gravity on the distribution of pulmonary ventilation. J. Appl. Physiol. 21: 778-784.

Buckingham, S., H. O. Heinemann, S. C. Sommers and W. F. McNary, Jr. 1966. Phospholipid synthesis in the large pulmonary alveolar cell: Its relation to lung surfactants. Am. J. Pathol. 48: 1027-1044.

Buist, S. A. 1975. The single-breath nitrogen test. New England J. Med. 293: 438-440.

Carter, A. and N. H. Martin. 1962. Serum sialic acid levels in health and disease. J. Clin. Pathol. 15: 69-72.

Carubelli, R. and M. J. Griffin. 1967. Sialic acid in Hela cells: Effect of hydrocortisone. Science 157: 693-694.

Chandrasekhar, N., L. Warren, J. Osbahr and K. Laki. 1962. Role of sialic acid in fibrinogen. Biochim. Biophys. Acta 63: 337-339.

Chanock, R. M. 1965. Mycoplasma infections of man. New England J. Med. 273: 1199-1206, 1257-1264.

Chevalier, G. and A. J. Collet. 1972. In vivo incorporation of choline-^{3}H, leucine-^{3}H and galactose-^{3}H in alveolar type II pneumocytes in relation to surfactant synthesis. A quantitative radioautographic study in mouse by electron microscope. Anat. Rec. 174: 289-310.

Clamp, J. R. and J. V. Jones. 1968. The antigenicity of the oligo-saccharide units of a soluble glycoprotein. Clinica Chimica Acta 21: 165-169.

Clements, J. A. 1970. Pulmonary surfactant. Am. Rev. Resp. Dis. 101: 984-990.

Clements, J. A., E. S. Brown and R. P. Johnson. 1958. Pulmonary surface tension and the mucous lining of the lung: Some theoretical consid-erations. J. Appl. Physiol. 12: 262-268.

Clements, J. S., R. F. Hustead, R. P. Johnson and I. Gribetz. 1961. Pulmonary surface tension and alveolar stability. J. Appl. Physiol. 16: 444-450.

Cohen, G. and N. L. Somerson. 1967. Mycoplasma pneumoniae: Hydrogen peroxide secretion and its possible role in virulence. Ann. N.Y. Acad. Sci. 143: 85-87.

Colacicco, G., A. R. Buckelew, Jr. and E. M. Scarpelli. 1973. Protein and lipid fractions of lung washings: Chemical characterization. J. Appl. Physiol. 34: 743-749.

Comroe, J. H. 1974. Physiology of respiration. Year Book Medical Publishers Inc., Chicago, Illinois.

Comroe, J. H., R. E. Forster, A. B. Dubois, W. A. Briscoe and E. Carlsen. 1962. The lung, clinical physiology and pulmonary function tests. Year Book Medical Publishers Inc., Chicago, Illinois.

Cook, G. M. 1968. Glycoproteins in membrane. Biol. Rev. 43: 363-389.

Coppola, J. A. and J. L. Ball. 1966. Uterine sialic acid in relation to ovarian steroids. Steroids 8: 345-352.

Crosfill, M. L. and J. G. Widdicombe. 1961. Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. J. Physiol. 158: 1-14.

Davidson, J. T., K. Wasserman, G. A. Lillington and R. W. Schmidt. 1966. Effect of aging on respiratory mechanics and gas exchange in rabbits. J. Appl. Physiol. 21: 837-842.

Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404-427.

Davis, M. M. and J. E. Richmond. 1968. Effect of dietary protein on serum proteins. Am. J. Physiol. 215: 366-369.

Dollfuss, R. E., J. Milic-Emili and D. V. Bates. 1967. Regional ventilation of the lung studied with boluses of [133]Xenon. Resp. Physiol. 2: 234-246.

Dubin, S. 1970. Lung compliance of normal unanesthetized beagle dogs. J. Vet. Res. 31: 895-902.

Dubin, S. E., A. J. Wilson and R. C. Pfleger. 1971. Changes in respiratory function of beagle dogs given pulmonary lavage. Am. J. Vet. Res. 32: 2033-2038.

Dugan, F. A., B. Radhakrishnamurthy, R. A. Rudman and G. S. Berenson. 1968. Stimulation of synthesis of glycoprotein enzymes in the rat uterus by estradiol. J. Endocrinol. 42: 261-266.

Edward, D. G. and E. A. Freundt. 1969. Classification of the myco-
plasmatales. Pages 147-200 in L. Hayflick, ed. The mycoplasmatal
and the L-phase of bacteria. Appleton-Century-Croft, New York.

Engen, R. L. 1970. Effect of steroids on serum sialic acids, values in
the canine. Proc. Soc. Exp. Biol. Med. 135: 778-780.

Engen, R. L. 1971. Serum sialic acid values in dogs with canine dis-
temper. Am. J. Vet. Res. 32: 803-804.

Engen, R. L., A. Anderson and L. L. Rouze. 1974. Automated procedure for
determination of sialic acids and 2-deoxyribose in blood and tissues.
Clin. Chem. 20: 1125-1127.

Feigl, E. O. and L. G. D'Alecy. 1972. Normal arterial blood pH, oxygen,
and carbon dioxide tensions in unanesthetized dogs. J. Appl.
Physiol. 32: 152-153.

Fenn, W. O. 1951. Mechanics of respiration. Am. J. Med. 10: 77-84.

Ferris, B. G., J. Mead and L. H. Opie. 1964. Partitioning of respiratory
flow resistance in man. J. Appl. Physiol. 19: 653-658.

Forster, R. E. 1957. Exchange of gas between alveolar air and pulmonary
capillary blood; pulmonary diffusing capacity. Physiol. Rev. 37:
391-452.

Forster, R. E. 1974. Pulmonary ventilation and blood gas exchange.
Pages 371-392 in W. A. Sodeman, Jr. and W. A. Sodeman, eds. Patho-
logic physiology mechanisms of disease. W. B. Saunders Co.,
Philadelphia.

Frosolono, M. F., B. L. Charms, R. Pawlowski and S. Slivka. 1970. Isola-
tion, characterization, and surface chemistry of a surface-active
fraction from dog lung. J. Lipid Res. 11: 439-457.

Fry, D. L., W. W. Stend, R. V. Ebert and R. I. Lubin. 1952. The measure-
ment of intraesophageal pressure and its relationship to intra-
thoracic pressure. J. Lab. Clin. Med. 40: 664-673.

Fuller, W. and R. L. Engen. 1971. Plasma sialic acid levels in normal
beagles. Iowa State Vet. 31: 26-28.

Galdston, M. and D. O. Shah. 1970. Lipid-protein association in lung
surfactant. Adv. Exp. Med. Biol. 7: 261-274.

Galdston, M., D. O. Shah and G. Y. Shinowara. 1969. Isolation and characterization of lung lipoprotein surfactant. J. Colloid and Interface Sci. 29: 319-334.

Ganong, W. F. 1975. Review of medical physiology. Long Medical Publications, Los Altos, California.

Gelb, A. F. and N. Zamel. 1975. Effect of aging on lung mechanics in healthy nonsmokers. Chest 65: 538-541.

Gesner, B. and L. Thomas. 1965. Sialic acid binding sites: Role in hemagglutination by Mycoplasma gallisepticum. Science 151: 590-591.

Gil, J. and O. K. Reiss. 1973. Isolation and characterization of lamellar bodies and tubular myelin from rat lung homogenates. J. Cell Biol. 68: 152-571.

Gillespie, D. J. and R. E. Hyatt. 1974. Respiratory mechanics in the unanesthetized dog. J. Appl. Physiol. 36: 98-102.

Glossmann, H. and D. M. Neville, Jr. 1971. Glycoproteins of cell surfaces. J. Biol. Chem. 246: 6339-6346.

Gluck, L., M. V. Kulovich, R. C. Borer, Jr., H. P. Brenner, G. H. Anderson and W. N. Spellacy. 1971. Diagnosis of the respiratory distress syndrome by amniocentesis. Am. J. Obstet. Gynecol. 109: 440-445.

Goco, R. V., M. B. Kress and O. C. Brantigan. 1963. Comparison of mucous glands in the tracheobronchial tree of man and animals. Ann. N.Y. Acad. Sci. 106: 551-571.

Gold, W. M., H. S. Kaufman and J. A. Nadel. 1967. Elastic recoil of the lungs in chronic asthmatic patients before and after therapy. J. Appl. Physiol. 23: 433-438.

Goodwin, R. F. W., A. P. Pomeroy and P. Whittlestone. 1965. Production of enzootic pneumonia in pigs with mycoplasma. Vet. Rec. 77: 1247-1249.

Goodwin, R. F. W., P. A. Pomeroy and P. Whittlestone. 1967. Characterization of Mycoplasma hyopneumoniae: Mycoplasma causing enzootic pneumonia of pigs. J. Hygiene 65: 85-96.

Goodwin, R. F. W., R. G. Hodgson, P. Whittlestone and R. L. Woodhams. 1969. Immunity in experimentally induced enzootic pneumonia of pigs. J. Hygiene 67: 193-208.

Gottschalk, A. 1956. The linkage of sialic acid in mucoprotein. Biochim. Biophys. Acta 20: 560-561.

Gottschalk, A. 1960. Correlation between composition, structure, shape and function of salivary mucoprotein. Nature (London) 186: 949-951.

Gottschalk, A. 1966. Glycoproteins and viruses. Pages 337-359 in R. Jeanloz and E. Balozs, eds. Metabolism and interactions. Academic Press, Inc., New York.

Gottschalk, A. and P. E. Lind. 1949. Product of interaction between influenza virus enzyme and ovomucin. Nature (London) 164: 232-233.

Haff, R. F. and R. C. Stewart. 1965. Role of sialic acid receptors in adsorption of influenza virus to chick embryo cells. J. Immunol. 94: 842-851.

Hamlin, R. L. and C. R. Smith. 1967. Characteristics of respiration in healthy dogs anesthetized with sodium pentobarbitol. Am. J. Vet. Res. 28: 173-178.

Heinemann, H. O. and A. P. Fishman. 1969. Non-respiratory functions of mammalian lung. Physiol. Rev. 49: 1-47.

Hirst, G. K. 1941. The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus. Science 94: 22-23.

Hodges, R. T., A. O. Betts and A. R. Jennings. 1969. Production of pneumonia in gnotobiotic pig with pure cultures of Mycoplasma hyopneumoniae. Vet. Rec. 84: 268-273.

Hogg, J. C., P. T. Macklem and W. M. Thurlbeck. 1968. Site and nature of airway obstruction in chronic obstructive lung disease. New England J. Med. 278: 1355-1360.

Hogg, J. C., P. T. Macklem and W. M. Thurlbeck. 1969. Resistance to collateral flow in excised human lungs. J. Clin. Invest. 48: 421-431.

Hogg, J. C., J. B. Agarawal, A. J. S. Gardiner, W. H. Palmer and P. T. Macklem. 1972. Distribution of airway resistance with developing pulmonary edema in dogs. J. Appl. Physiol. 32: 20-26.

Holland, J., J. Milic-Emili, P. T. Macklem and D. V. Bates. 1968. Regional distribution of pulmonary ventilation and perfusion in elderly subjects. J. Clin. Invest. 47: 81-92.

Hyatt, R. E. and R. E. Flath. 1966. Influence of lung parenchyma on pressure-diameter behaviour of dog bronchi. J. Appl. Physiol. 21: 1448-1452.

Hyatt, R. E. and R. E. Wilcox. 1961. Extrathoracic airway resistance in man. J. Appl. Physiol. 16: 326-330.

Jericho, N. W. F. 1968. Pathogenesis of pneumonia in pigs. Vet. Rec. 82: 507-520.

Kabat, E. A. 1956. Blood group substances: Their chemistry and immuno-chemistry. Academic Press, New York.

Kaneko, K., J. Milic-Emili, M. B. Dolovich, A. Dawson and D. V. Bates. 1966. Regional distribution of ventilation and perfusion as a function of body position. J. Appl. Physiol. 21: 767-777.

Kennedy, T. G. and C. W. Emmens. 1975. Effects of estrogen and pro-gesterone on uterine sialic acid in ovariectomized rats. Steroid 25: 285-295.

Kikkawa, Y., E. K. Motoyama and C. D. Cook. 1965. The ultrastructure of the lungs of lambs. The relation of osmiophilic inclusions and alveolar lining layer to fetal maturation and experimentally pro-duced respiratory distress. Am. J. Pathol. 47: 877-881.

Kikkawa, Y., Y. Kokichi, F. Smith, B. Packard and K. Suzuki. 1975. The type II epithelial cells of the lung. II. Chemical composition and phospholipid synthesis. Lab. Invest. 32: 295-302.

King, R. J. 1974. The surfactant system of the lung. Fed. Proc. 33: 2238-2247.

King, R. J., D. J. Klass, E. G. Gikas and J. A. Clements. 1973. Isola-tion of apoproteins from canine surface active material. Am. J. Physiol. 224: 788-795.

King, R. J., J. Ruch, E. G. Gikas, A. G. Platzker and R. K. Creasy. 1975. Appearance of apoproteins of pulmonary surfactant in human amniotic fluid. J. Appl. Physiol. 39: 735-738.

Klein, R. M. and S. Margolis. 1968. Purification of pulmonary sur-factant by ultracentrifugation. J. Appl. Physiol. 25: 654-658.

Knutsen, G. L. 1976. The influence of age, sex, and weight on respira-tory function in the sedate canine. M.S. thesis. Iowa State University, Ames.

Kontas, R. V. and M. E. Avery. 1971. Accelerated appearance of pulmonary surfactant in the fetal rabbit. J. Appl. Physiol. 30: 358-361.

140

Kraemer, P. M. 1966. Sialic acid in mammalian cell lines. J. Cell Physiol. 67: 23-34.

Kuhn, C., F. Gyorkey, B. E. Levine and R. J. Romirez. 1966. Pulmonary alveolar proteinosis. Lab. Invest. 15: 492-509.

Lam, K. M. C. 1970. Serologic and immunologic studies on Mycoplasma hyopneumoniae pneumonia of swine. Ph.D. thesis. Iowa State University, Ames.

Lam, K. M. and W. P. Switzer. 1971. Mycoplasmal pneumonia of swine: Development of an indirect hemagglutination test. Am. J. Vet. Res. 32: 1731-1741.

Lambson, R. O. and J. E. Cohn. 1968. Ultrastructure of the lung of the goose and its lining of surface material. Am. J. Anat. 122: 631-635.

Lannek, H. and S. Bornfors. 1957. Immunity to enzootic pneumonia in pigs following recovery from the disease. Nordisk Veterinarmed. 9: 91-98.

Leblanc, P., F. Ruff and J. Milic-Emili. 1970. Effects of age and body position on "airway closure" in man. J. Appl. Physiol. 28: 448-451.

L'Ecuyer, C. 1969. Enzootic pneumonia in pigs: Propagation of a causative mycoplasma in cell cultures and in artificial medium. Can. J. Comp. Med. 33: 10-19.

L'Ecuyer, C. and W. P. Switzer. 1963. Virus pneumonia of pigs: Attempts at propagation of the causative agent in cell cultures and chicken embryos. Can. J. Comp. Med. Vet. Sci. 27: 91-99.

Levinson, B., D. Pepper and G. Belyavin. 1969. Substituted sialic acid prosthetic groups as determinants of viral hemagglutination. J. Virol. 3: 477-485.

Licht, P. and H. Papkoff. 1972. Relationship of sialic acid to the biological activity of vertebrate gonadotropins. Gen. Comp. Endocrinol. 19: 102-114.

Liggins, G. C. 1969. Premature delivery of fetal lambs infused with glucocorticoids. J. Endocrinol. 45: 515-523.

Livingston, C. W., E. L. Stair, N. R. Underdahl and C. A. Mebus. 1972. Pathogenesis of mycoplasmal pneumonia in swine. Am. J. Vet. Res. 33: 2249-2258.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951.
Protein measurement with the folin phenol reagent. J. Biol. Chem.
193: 265-275.

MacFarland, H. N. 1976. Respiratory toxicology. Essential Toxicology
7: 144-153.

Macklem, P. T. and J. Mead. 1967. Resistance of central and peripheral
airways measured by a retrograde catheter. J. Appl. Physiol. 22:
395-401.

Macklem, P. T., A. J. Woolcock, J. C. Hogg, J. A. Nadel and N. J. Wilson.
1969. Partitioning of pulmonary resistance in the dog. J. Appl.
Physiol. 26: 798-805.

Macklin, C. C. 1954. The pulmonary alveolar mucoid film and the pneumon-
ocytes. Lancet I: 1099-1104..

Maré, C. J. 1965. Characterization of causative agent of virus pneumonia
of pig (VPP). Ph.D. thesis. Iowa State University, Ames.

Maré, C. J. and W. P. Switzer. 1965. New species: Mycoplasma hyopneu-
moniae a causative agent of virus pig pneumonia. Vet. Med./Small
Animal Clin. 60: 841-846.

Margoliash, E., J. R. Shenk, M. P. Hargi, S. Burshas, W. R. Richter,
G. H. Barlow and A. A. Moscona. 1965. Characterization of specific
cell aggregating materials from sponge cells. Biochem. Biophys.
Res. Commun. 20: 383-388.

Massaro, G. D. and D. Massaro. 1972. Granular pneumocytes. Electron
microscopic radioautographic evidence of intracellular protein
transport. Am. Rev. Resp. Dis. 105: 927-931.

Mauderly, J. L. 1968. A comparison of the pulmonary function of immature,
young adult, and aged beagle dogs. Finion Product Inhalation Program
Annual Report. Lovelace Foundation for Medical Education and Re-
search, Albuquerque, New Mexico.

McCarthy, D. D., R. Spencer, R. Greene and J. Milic-Emili. 1972. Measure-
ment of "closing volume" as a simple and sensitive test for detection
of small airway disease. Am. J. Med. 52: 747-753.

McClelland, D. A. and J. M. Bridges. 1973. The total N-acetyl neurominic acid content of human normal and lymphatic leukaemic lymphocytes. Br. J. Cancer 27: 114-119.

Mead, J. 1961. Mechanical properties of lungs. Physiol. Rev. 41: 281-330.

Melnykovych, G. and C. F. Bishop. 1967. Specificity of prednisolone effect on cell volume, RNA and protein in cell line inducible and noninducible alkaline phosphatase. Endocrinology 81: 251-255.

Milic-Emili, J., J. Mend, J. M. Turner and E. M. Glauser. 1964. Improved technique for estimating pleural pressure from esophageal balloons. J. Appl. Physiol. 1-: 207-211.

Milic-Emili, J., J. A. M. Henderson, M. B. Dolovich, D. Trop and K. Komeko. 1966. Regional distribution of inspired gas in the lung. J. Appl. Physiol. 21: 749-759.

Mitten, C., N. H. Edelman, A. H. Norris and N. W. Shock. 1965. Relationship between chest wall and pulmonary compliance and age. J. Appl. Physiol. 20: 1211-1216.

Morrison, W. R. 1964. A fast, simple and reliable method for the microdetermination of phosphorus in biological materials. Anal. Biochem. 7: 218-224.

Morrison, W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600-608.

Mousa, W., D. R. Ten Eyck and C. I. Waslien. 1973. Serum glycoproteins and malaria in Ethiopia. J. Lab. Clin. Med. 82: 924-929.

Murphy, S. G., A. S. Kainer and D. F. Clyde. 1972. Characterization and pathophysiology of serum glycoprotein alterations in malaria. J. Lab. Clin. Med. 79: 55-61.

Naden, A. H. 1967. Bronchiolar and large alveolar cell in pulmonary phospholipid metabolism. Science 158: 1323-2324.

Neergard, K. von. 1929. Neue Auffassungen uber einen Grundbegriff der Atemmechanic. Die Retraktionskraft der Lunge, abhonzig von der Oberflachensponsung in der Alveolen. Z. Ges. Exptl. Med. 66: 372-394.

Oboegbulem, S. I. 1973. Response of swine to Mycoplasma hyopneumoniae vaccination. M.S. thesis. Iowa State University, Ames.

Ogilvie, C. M. R. E. Forster and W. S. Blakemore. 1957. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lungs for carbon monoxide. J. Clin. Invest. 36: 1-17.

Oppenheimer, S. B., M. Edidin, C. W. Orr and S. Roseman. 1969. An L-glutamine requirement for intercellular adhesion. Proc. Nat. Acad. Sci. USA 63: 1395-1402.

Ostle, B. and R. W. Mensing. 1975. Statistics in research. 3rd ed. Iowa State University Press, Ames.

Otis, A. B. 1954. The work of breathing. Physiol. Rev. 34: 449-458.

Page-Roberts, B. A. 1972. Preparation and partial characterization of a lamellar body function from rat lung. Biochim. Biophys. Acta 260: 334-338.

Papkoff, H. 1965. Some biological properties of a potent follicle-stimulating hormone preparation. Acta Endocrinol. 48: 439-445.

Papkoff, H. 1966. Recent studies on the purification and properties of ovine, bovine and human interstitial cell-stimulating hormone (ICSH, LH) and ovine follicle-stimulating hormone (FSH). Excerpta Med. Int. Congr. Ser. 112: 334-339.

Patterson, M. K., M. D. Maxwell and T. A. McCoy. 1963. Influence of secondary infection on tumor host serum-bond carbohydrates. Proc. Soc. Exp. Biol. Med. 113: 689-691.

Pattison, J. H. 1956. A histological study of a transmissible pneumonia of pigs characterized by extensive lymphoid hyperplasia. Vet. Rec. 38: 490-496.

Pattle, R. E. 1958. Properties, function and origin of the alveolar lining layer. Proc. Roy. Soc. London Ser. B 148: 217-240.

Pattle, R. E. 1965. Surface lining of lung alveoli. Physiol. Rev. 45: 48-79.

Patwardham, V. N., R. H. Maghrabi, W. Mousa, M. K. Gabs and S. El-Maraghy. 1971. Serum glycoproteins in protein-caloric deficiency disease. Am. J. Clin. Nutr. 24: 906-912.

Pawlowski, R., M. F. Frosolono, B. L. Charms and R. Przybylski. 1971. Intra and extracellular compartmentalization of the surface-active fraction in dog lung. J. Lipid Res. 12: 538-544.

Pfleger, R. C. and H. G. Thomas. 1971. Beagle dog pulmonary surfactant lipids. Arch. Internat. Med. 127: 863-872.

Pfleger, R. C., R. F. Henderson and J. Waide. 1972. Phosphatidylglycol: A major component of pulmonary surfactant. Chem. Phys. Lipids 9: 51-68.

Pickrell, J. A., J. L. Mauderly, B. A. Muggenburg and V. C. Luft. 1974. Influence of fasting on blood gas tension, pH, and related values in dog. Am. J. Vet. Med. 34: 805-808.

Pickrell, J. A. and S. J. Schluter. 1973. The rapid processing of canine blood gas tension and pH measurements. Am. J. Vet. Res. 34: 95-100.

Pickrell, J. A., S. E. Dubin and J. C. Elliott. 1971. Normal respiratory parameters of unanesthetized beagle dogs. Lab. Anim. Sci. 21: 677-679.

Pierce, J. A. and R. V. Ebert. 1958. The barrel deformity of the chest, the senile lung and obstructive pulmonary emphsema. Am. J. Med. 25: 13-22.

Pierce, J. A. and R. V. Ebert. 1959. The elastin properties of the lung collagen and elastin. Am. Rev. Resp. Dis. 80: 45-49.

Pierce, J. A. and J. B. Hocott. 1960a. Studies on the collagen and elastin content of the human lung. J. Clin. Invest. 39: 8-14.

Pierce, J. A. and J. B. Hocott. 1960b. Studies of lung collagen and elastin. Am. Rev. Resp. Dis. 80: 45-50.

Rahn, H. and L. E. Farhi. 1964. Ventilation, perfusion and gas exchange - the VA/Q concept. Pages 735-765 in W. O. Fenn and H. Rahn, eds. Handbook of Physiology. American Physiological Society, Washington, D.C.

Rankin, J., R. S. McNeill and R. E. Forster. 1961. The effect of anemia on the alveolar capillary exchange of carbon monoxide in man. J. Clin. Invest. 40: 1323-1330.

Rajalakshmi, M., M. R. N. Prasad and S. Mohla. 1969. Effect of estrogen and clomiphene on uterine sialic acid in the rat during delayed implantation. Steroids 14: 47-53.

Roberts, D. H. 1968. Serological diagnosis of Mycoplasma hyopneumoniae infection in pigs. Vet. Rec. 82: 362-363.

Roberts, D. H. 1973. Preliminary studies on the cell-mediated immune response in pigs to Mycoplasma hyopneumoniae. Br. Vet. J. 129: 427-438.

Roberts, D. H. and T. W. A. Little. 1970. Serological studies in pigs with Mycoplasma hyopneumoniae infection in pigs. J. Comp. Pathol. 80: 211-220.

Robinson, N. E. and J. R. Gillespie. 1970. Pulmonary function testing as an aid to the diagnosis of pulmonary disease in the dog. Twentieth Gaines Veterinary Symposium.

Robinson, N. E. and J. R. Gillespie. 1972. Lung mechanics in aging beagle dogs from a closed colony. Fed. Proc. 31: 355. (Abstr.)

Robinson, N. E. and J. R. Gillespie. 1973. Lung volumes in aging beagle dogs. J. Appl. Physiol. 35: 317-321.

Rombourg, A. and C. P. Leblond. 1967. Electron microscope observations on the carbohydrate-rich cell count present at the surface of cells in the rat. J. Cell Biol. 32: 27-53.

Rosen, S. H., B. Castleman and A. A. Liebow. 1958. Pulmonary alveolar proteinosis. N. England J. Med. 258: 1123-1142.

Ruppel, G. 1975. Manual of pulmonary function testing. C. V. Mosby Co., St. Louis, Missouri.

Scarpelli, E. M. 1968a. Lung surfactant: Dynamic properties, metabolic pathways, and possible significance in the pathogenesis of the respiratory distress syndrome. Bull. N.Y. Acad. Med. 44: 431-445.

Scarpelli, E. M. 1968b. The surfactant system of the lung. Lea and Febiger, Philadelphia.

Scarpelli, E. M., B. C. Clutario and F. A. Taylor. 1967. Preliminary identification of the lung surfactant system. J. Appl. Physiol. 23: 880-886.

Scarpelli, E. M., G. Colacicco and S. J. Chang. 1971. Significance of methods for isolation and characterization of pulmonary surfactants. Resp. Physiol. 12: 179-198.

Seal, U. S. and R. P. Doe. 1962. Corticosteroid-binding globulin isolation from plasma of diethylstilbestrol-treated men. J. Biol. Chem. 237: 3136-3140.

Shear, M. and A. G. E. Pearse. 1963. A direct histochemical method for the demonstration of sialic acid. Nature 198: 1273-1275.

Shetlar, M. R. 1961. The serum glycoproteins, their origin and significance. Ann. N.Y. Acad. Sci. 94: 44-53.

Singh, R. and B. Ramraju. 1967. Serum sialic acid values in healthy Indian adults. Ind. J. Med. Res. 55: 267-269.

Singh, R., B. K. Sur, S. N. Agarwal and B. Ramraju. 1967. Serum sialic acid in malignancy. Ind. J. Med. Res. 55: 270-272.

Sinha, A. K. and G. Melnykovych. 1972. Sialic acid in prednisolone treated Hela cells; suggested mechanism for elevated levels at the cell surface. Biochem. Biophys. Res. Commun. 49: 984-991.

Sinha, A. K. and G. Melnykovych. 1973. Repression of sialopeptidase activity in Hela cells by prednisolone. J. Biol. Chem. 248: 7332-7335.

Slavik, M. F. and W. P. Switzer. 1972. Development of a microtitration complement-fixation test for diagnosis of mycoplasmal swine pneumonia. Iowa State J. Res. 47: 117-128.

Snyder, S. and G. Ashwell. 1971. Quantitation of specific serum glycoproteins in malignancy. Clinica Chimica Acta 34: 449-455.

Sobeslavsky, O., B. Prescott and R. M. Channock. 1968. Adsorption of Mycoplasma pneumoniae to neurominic acid receptors of various cells and possible role in virulence. J. Bacterol. 96: 695-705.

Spiro, R. G. 1963. Glycoproteins, structure, metabolism and biology. New England J. Med. 269: 566-573, 616-621.

Springer, G. F. and N. J. Ansell. 1958. Inactivation of human erythrocyte agglutinogens M and N by influenza viruses and receptor-destroying enzyme. Proc. Nat. Acad. Sci. USA 44: 183-188.

Stahl, W. R. 1967. Scaling of respiratory variables in mammals. J. Appl. Physiol. 22: 453-460.

Svennerholm, L. 1958. Quantitative estimation of sialic acids. Acta Chem. Scand. 12: 547-554.

Switzer, W. P. 1969. Page 607 in L. Hayflick, ed. Mycoplasmatals and the L-phase of bacteria. North-Holland Publishing Co., Amsterdam.

Switzer, W. P. and K. S. Preston. 1974. Application of the complement-fixation test to the control of Mycoplasma pneumoniae in swine. 111th Annual Meeting, American Veterinary Medicine Association (Abstr.)

Switzer, W. P. and R. F. Ross. 1975. Mycoplasmal diseases. In H. W. Dunne and A. D. Leman, eds. Diseases of swine. 4th ed. Iowa State University Press, Ames.

Takatori, I. 1969. Demonstration of complement fixation antibody against Mycoplasma hyopneumoniae in Japan. Nat. Inst. Anim. Hlth. Quart. 9: 183-184.

Tierney, D. F., J. A. Clements and H. J. Trahan. 1967. Rates of replacement of lecithins and alveolar instability in rat lungs. Am. J. Physiol. 213: 671-676.

Tobiska, J., Z. Brada and B. Gustofsson. 1969. Influence of sterile turpentine-oil inflammation on concentration of serum glycoprotein in germ free rats. Life Sci. 8: 949-951.

Turner, J. M., J. Mead and M. E. Wohl. 1968. Elasticity of human lungs in relation to age. J. Appl. Physiol. 25: 664-671.

Van Herick, W. and M. D. Eaton. 1945. An unidentified pleuropneumonia-like organism isolated during passages in chick embryos. J. Bacteriol. 50: 47-55.

Vesselinovitch, S. D. 1955. Electrophoretic studies of oedema disease in swine. Br. Vet. J. 111: 398-403.

Wang, N. S., R. V. Kotas, M. E. Avery and W. M. Thurlbeck. 1971. Accelerated appearance of osmiophilic bodies in fetal lungs following steroid injection. J. Appl. Physiol. 30: 362-365.

Wardi, A. H. and G. A. Michos. 1972. Alcian blue staining of glycoproteins in acrylamide disc electrophoresis. Anal. Biochem. 49: 607-609.

Warren, L. 1959. The thiobarbituric assay of sialic acids. J. Biol. Chem. 324: 1971-1975.

Weimer, H. E. and C. Humelbough. 1965. Alterations in protein-bound carbohydrate levels of rat serum during food restriction and re-feeding. Proc. Soc. Biol. Exp. Med. 118: 842-844.

West, J. B. 1974. Respiratory physiology - the essentials. Williams and Wilkins Co., Baltimore, Maryland.

Widdicombe, J. G. and J. A. Nadel. 1963. Airway volume, airway resistance, and work and force of breathing: Theory. J. Appl. Physiol. 18: 863-868.

Williams, M. H., Jr., N. S. Seriff, T. Akyol and O. H. Yoo. 1961. The differing capacity of the lung in acute pulmonary tuberculosis. Am. Rev. Resp. Dis. 84: 814-820.

Williams, R. and P. Hugh-Jones. 1960. The significance of lung function changes in asbestosis. Thorax 15: 109-112.

Wilson, A. F., R. D. Fairshter, J. R. Gillespie and J. Hackney. 1976. Evaluation of abnormal lung function. Ann. Rev. Pharmacol. and Toxicol. 16: 465-486.

Winzler, R. J. 1955. Determination of serum glycoproteins. Methods Biochem. Anal. 2: 279-311.

Woolcock, A. J. and P. T. Macklem. 1971. Mechanical factors influencing collateral ventilation in human, dog, and pig lungs. J. Appl. Physiol. 30: 99-115.

Woolcock, A. J. and J. Mead. 1968. The static elastic properties of the lungs in asthma. Am. Rev. Resp. Dis. 98: 788-796.

Young, G. A. 1964. Virus pneumonia of pigs. In H. W. Dunne, ed. Diseases of swine. 2nd ed. Iowa State University Press, Ames.

Young, G. A. and N. R. Underdahl. 1955. An evaluation of influenza in midwestern swine. Am. J. Vet. Res. 16: 545-552.

Young, G. A. and N. R. Underdahl. 1960. Certification of swine herds as virus pneumonia-free. J. Am. Vet. Med. Assoc. 137: 186-189.

Young, S. L. and D. F. Tierney. 1972. Dipalmitoyl lecithin secretion and metabolism by the rat lung. Am. J. Physiol. 222: 1539-1544.

Zardini, P. and J. B. West. 1966. Topographical distribution of ventilation in isolated lung. J. Appl. Physiol. 21: 794-802.

Zilliken, F., G. H. Werner, R. K. Silver and P. Gyorgy. 1957. Studies ob the enzymatic properties of influenza virus. I. The action of influenza B virus and RDE on the hemagglutination inhibitor of human meconium. Virology 3: 464-474.

Zimmerman, D. R., M. L. Spear and W. P. Switzer. 1971. Protein, Mycoplasma hyopneumoniae and ascaris in pigs. J. Anim. Sci. 33: 241.

ACKNOWLEDGMENTS

APPENDIX A: ABBREVIATIONS, DEFINITIONS, AND REAGENTS
FOR GLYCOPROTEIN ELECTROPHORESIS

151

Abbreviations

Parameter	Abbreviation	Unit
Weight	Wt	Kg
Heart rate	HR	beats/min
Respiratory rate	f	breaths/min
Tidal volume	V_T	ml
Minute volume	MV	ml/min
Expiratory velocity	$\dot{V}_E$	L/min
Inspiratory velocity	$\dot{V}_I$	L/min
Esophageal pressure	P_E	CmH_2O
Lung compliance	Cdyn	ml/cmH_2O
Airway resistance	R_A	$CmH_2O/L/sec$
Work/kg	WK/kg	g-m/kg
Work/minute/kg	Wk/min/kg	g-m/min/kg
Partial pressure of arterial O_2	PaO_2	mmHg (Torr)
Partial pressure of arterial CO_2	$PaCO_2$	mmHg (Torr)
pH	pH	pH
hemoglobin	Hb	gm/100 ml
Hematocrit	PCV	percent

Definitions

The definition of the respiratory parameters are defined by Comroe et al. (1962).

Tidal volume (V_T) - The depth of breathing is the volume of gas inspired or expired during each respiratory cycle.

Inspiratory reserve volume (IRV) - The maximal volume that can be inspired from end-tidal inspiration.

Expiratory reserve volume (ERV) - The maximal volume that can be expired from resting expiratory level.

Residual volume (RV) - The volume of gas in lungs at end of maximal expiration.

Total lung capacity (TLC) - The volume of gas in lung at end of maximal inspiration.

Vital capacity (VC) - The maximal volume that can be expired after maximal inspiration.

Inspiratory capacity (IC) - The maximal volume that can be inspired from resting expiratory level.

Functional residual capacity (FRC) - The volume of gas in lungs at resting expiratory level.

Airway resistance (R_A) - The pressure between the airway opening (i.e., mouth or nose) and the alveoli, in relation to simultaneous air flow; expressed as CmH_2O/liter/sec.

Alveolar gas - Expired gas that has come from alveoli. The definition of mean alveolar gas concentration is complicated by the discontinuous

nature of lung ventilation and perfusion, and by the nonuniform behavior of the lung in regard to these aspects of function.

Alveolar ventilation - If the lungs behaved as a completely uniform system, alveolar ventilation could be defined as the tidal volume minus the anatomical dead-space volume, multiplied by the respiratory frequency. In many situations, however, alveolar ventilation can be defined only in terms of the arterial PCO_2, the level of which ordinarily reflects the total effective alveolar ventilation.

Blood-gas tension - The pressure in mmHg of a gas in the blood. Note that pressures between a liquid and a gas must always be in equilibrium, regardless of solubility, buffer systems, partition coefficients, or dissociation curves.

Compliance, dynamic (C dyn) - The ratio of the tidal volume to the difference in pressure at points of zero gas flow, expressed in ml/cmH_2O.

Dead space, anatomical (inert-gas dead space) - The volume of all nongas-exchanging passages in the lung, normally comprising the upper airway and bronchial tree as far as the respiratory bronchioles.

Work of breathing - The cumulative product of instantaneous pressure developed by the respiratory muscles and volume of air moved in a breathing cycle, expressed as g-m/kg.

Reagents for Glycoprotein Electrophoresis

Buffer

Glycine-NaOH buffer pH 10.6 (stock buffer was diluted 8 times 0.05 M

for electrophoresis)

364 ml of 1.0 M NaOH

400 ml of 1.0 M glycine

QS to 1 liter for 0.4 M stock buffer

Gel

Sol. "A" Acrylamide 30.0 grams

N,N'-methylene-bis-acrylamide 1.0 g were suspended in 123 ml

H_2O. Mix repeatedly until most solid is in solution. A clear

solution was obtained after filtration through a glass wool

plug.

Sol. "B" Stock buffer (0.4 M) indicated above, stored at 5°C.

Sol. "C" (TEMED) N,N,N',N' - tetramethyl-ethylenediamine 1% sol. 1 ml in

100 ml H_2O.

Sol. "D" Aqueous ammonium persulfate solution (0.56% w/v).

The gels containing 5% acrylamide were obtained by adding the following

reagents in the order indicated to 6 ml H_2O in a beaker:

4.0 ml Sol. "A"
2.0 ml Sol. "B"
2.0 ml Sol. "C"
2.0 ml Sol. "D"

After adding Sol. "D", polymerization was usually complete in 15 minutes.

APPENDIX B: THE ANALYSIS OF VARIANCE PLAN,

AND OBSERVED MEAN SQUARES DATA

Table B1. The percent of plasma protein fractions, and total plasma protein in control and infected pigs

	Albumin (%)	Alpha α_1 (%)	Alpha α_2 (%)	Beta β (%)	Gamma γ_1 (%)	Gamma γ_2 (%)	Total plasma protein** (gm %)
4th week of infection							
Control (4)[a]	49.17±1.82[b]	5.42±0.72	7.62±0.80	8.80±0.87	7.78±0.69	21.15±2.03	5.65±0.21
Infected (6)	36.45±1.65**	5.82±0.30	10.27±0.77	10.06±0.49	9.84±0.74	27.56±1.48*	6.33±0.04
5th week of infection							
Control (4)	51.08±2.71	4.07±0.35	10.21±1.22	11.45±1.04	7.53±1.49	15.67±1.54	5.25±0.13
Infected (6)	29.53±2.11**	8.39±1.37*	11.33±0.88	11.98±1.03	8.72±1.13	30.79±2.07**	6.40±0.10
6th week of infection							
Control (5)	46.09±1.30	6.64±0.83	9.88±1.13	8.95±0.83	7.46±0.80	20.99±1.60	5.42±0.17
Infected (7)	22.68±1.46**	7.41±0.62	10.91±0.94	10.99±0.47*	12.29±1.68*	35.49±2.06**	6.53±0.10

[a]Number of observations.

[b]Values are means±SEM, expressed as % of total plasma protein fraction

*Significant at P<0.05.

**Significant at P<0.01.

Table B2. Fatty acid esters of sediment surfactant

	# carbon					
	14	14:1	16	16:1	18	18:1
4th week of infection						
Control (4)[a]	3.37±0.76[b]	2.96±0.74	70.34±2.12	6.96±1.83	6.00±0.84	11.13±1.52
Infected (6)	2.44±0.30	4.82±1.84	69.91±4.07	11.63±1.51	7.76±0.37	9.24±1.32
5th week of infection						
Control (4)	5.93±1.13	-	76.43±5.36	8.79±2.02	7.75±1.85	5.49±2.45
Infected (6)	3.47±0.62	2.52±0.22	73.51±4.67	10.68±3.03	7.44±0.74	6.79±1.08
6th week of infection						
Control (5)	4.50±0.79	2.72±0.98	76.65±5.06	6.34±3.65	3.42±0.92	7.23±1.67
Infected (7)	4.47±0.53	2.97±0.15	75.90±3.01	11.06±2.35	5.60±1.43	6.52±1.03

[a]Number of observations.

[b]Values are mean±SEM, expressed as % of total fatty acids.

Table B3. The percent composition of phospholipid fraction in the sediment surfactant and lung tissue

	Lecithin (%)	Ethanolamine (%)	Sphingomyelin lysolecithin (%)
4th week of infection			
Sediment surfactant			
Control (3)[a]	81.79±0.40[b]	7.88±0.21	10.33±0.32
Infected (4)	81.19±0.52	8.25±0.40	10.55±0.50
Lung tissue			
Control (4)	55.63±1.78	17.23±1.01	27.15±1.62
Infected (4)	53.91±1.76	20.02±1.63	25.80±3.05
5th week of infection			
Sediment surfactant			
Control (3)	79.07±1.58	9.44±0.83	11.48±0.80
Infected (4)	78.39±4.32	9.27±1.77	12.35±2.57
Lung tissue			
Control (4)	55.44±0.81	18.01±1.35	24.06±1.93
Infected (4)	54.83±0.72	19.87±1.06	25.31±0.99

[a]Number of observations.

[b]Values are mean±SEM, expressed as % of total phospholipid.

Table B4. Distribution of radioactivity between phospholipid fractions in sediment surfactant and lung tissue

	Lecithin (%)	Ethanolamine (%)	Sphingomyelin lysolecithin (%)
4th week of infection			
Sediment surfactant			
Control (3)[a]	90.34±0.47[b]	4.15±0.31	5.50±0.27
Infected (4)	89.59±0.45	4.01±0.75	6.42±0.36
Lung tissue			
Control (4)	72.34±1.28	10.39±1.83	17.29±1.02
Infected (4)	71.77±0.40	9.61±0.97	17.77±0.40
5th week of infection			
Sediment surfactant			
Control (3)	89.88±1.50	4.10±0.69	6.04±0.81
Infected (4)	88.28±1.20	3.67±0.40	8.05±1.00
Lung tissue			
Control (4)	64.25±1.72	11.72±0.70	24.04±1.23
Infected (4)	64.06±2.69	10.72±1.68	25.15±1.63

[a]Number of observations.

[b]Values are mean±SEM, expressed as % of total phospholipid.

Table B5. Analysis of variance plan and observed mean squares for body weight, body weight gain, body temperature, heart rate, respiratory rate, oxygen consumption

SV	df	Mean squares					
		Body wt	Body wt gain	Body temp.	HR	RR	O_2 cons.
Treatment	1	40.74	24490.96	0.04	20.44	0.20	26.17**
Period	2	88.07	94613.84**	0.25	4936.54	18.07	1.17
Treatment x period	2	18.85	1606.03	0.19	690.96	17.05	0.00
Error	26	12.72	4093.20	0.44	780.44	7.60	0.69
Total	31						

**Statistical significance is at $P<0.01$.

Table B6. Analysis of variance plan and observed mean squares for V_T, $\dot{V}_I$, $\dot{V}_E$, minute volume, alveolar ventilation, P_E, C dyn, R_A, WK', WK

SV	df	Mean squares			
		V_T	$\dot{V}_I$	$\dot{V}_E$	Minute volume
Treatment	1	30576.69**	162.30	404.52**	11307677.85**
Period	2	1850.70	9.28	5.99	1023794.10
Treatment x period	2	896.30	16.14	15.38	591633.80
Error	26	315.82	18.12	7.71	436310.74
Total	31				

**Statistical significance is at P<0.01.

	Mean squares				
Alveolar ventilation	P_E	Cdyn	R_A	WK_1	WK_2
14305174.28**	253.59**	300.14	386.03	0.029**	11.14**
1739749.10	8.14	5.70	77.37	0.004	1.48
521283.10	3.21	1.08	23.27	0.002	0.82
238108.66	4.51	20.10	32.35	0.0003	0.16

Table B7. Analysis of variance plan and observed mean squares for pH, PAO_2, $PACO_2$, HCO_3, BE, CO_2 content, AO_2, ACO_2

SV	df	Mean squares							
		pH	PAO_2	$PACO_2$	HCO_3	BE	CO_2 content	AO_2	ACO_2
Treatment	1	0.012	359.55	131.04	133.36	195.79**	157.18	598.07	501.98*
Period	2	0.001	18.20	30.46	14.68	17.27	19.01	324.12	101.92
Treatment x period	2	0.0001	3.56	0.00	0.00	0.00	0.00	203.96	54.42
Error	26	0.005	65.19	19.85	13.85	9.76	14.15	84.81	28.82
Total	31								

*Statistical significance is at $P<0.05$.

**Statistical significance is at $P<0.01$.

Table B8. Analysis of variance plan and observed mean squares for Hb, PCV, RBC, WBC, neutrophils, monocytes, eosinophils, basophils

SV	df	Mean squares								
		Hb	PCV	RBC	WBC	Neut.	Lymph.	Mono.	Eosin.	Baso.
Treatment	1	2.65	29.39	0.03	98.87	314.64	611.24	5.27	0.30	0.23
Period	2	8.97	24.-3	3.63	15.96	7.73	25.23	3.75	0.91	0.30
Treatment x period	2	0.23	7.63	0.26	3.50	21.08	14.19	0.62	1.45	0.00
Error	26	0.84	6.83	1.02	7.63	146.36	132.53	2.75	1.19	0.57
Total	31									

165

Table B9. Analysis of variance plan and observed mean squares for the amount of sediment, supernatant, and total lung surfactant from 1 liter of lavage fluid

SV	df	Mean squares Sediment	Supernatant	Total lung surfactant
Treatment	1	26208.95**	289341.25**	489714.86**
Period	2	2491.20	3868.06	167.16
Treatment x period	2	1198.70	2491.06	2878.96
Error	26	1041.87	8853.40	12220.91
Total	31			

**Statistical significance is at P<0.01.

Table B10. Analysis of variance plan and observed mean squares for percent of protein in sediment, supernatant, total lung surfactant, and plasma protein

| SV | df | Mean squares | | | |
		Sediment	Supernatant	Lung	Plasma protein
Treatment	1	56.29	5169.24**	0.17	7.53**
Period	2	7.55	193.49	1.28	0.05
Treatment x period	2	63.88	107.22	0.51	0.17
Error	26	37.86	48.05	0.76	0.08
Total	31				

**Statistical significance is at $P<0.01$.

Table B11. Analysis of variance plan and observed mean squares for percent total lipid of sediment, supernatant, total lung surfactant, and phospholipid content (mg/gm dry matter) of

SV	df	Mean squares				
		Sediment	Supernatant	Lung	Lipid-P Sediment	Lipid-P lung
Treatment	1	82.19	2397.34**	1.48	9618.04	970.06
Period	2	25.23	3.05	4.38	550.15	139.73
Treatment x period	2	57.93	4.72	1.61	1085.49	429.39
Error	26	41.59	33.59	4.31	1447.07	222.57
Total	31					

**Statistical significance is at $P<0.01$.

168

Table B12. Analysis of variance plan and observed mean squares for
sialic acid concentration in sediment, supernatant, total
pulmonary surfactant, lung tissue, plasma

SV	df	Sediment <.08	Supernatant	Total surfactant	Lung	Plasma
			Mean squares			
Treatment	1	195.92	9156.15**	12030.77**	0.01	88.99
Period	2	18.39	124.43	168.23	0.39	86.90
Treatment x period	2	35.14	125.90	298.33	0.14	23.89
Error	26	13.75	306.83	371.14	0.17	49.70
Total	31					

**Statistical significance is at $P<0.01$.

Table B13. Analysis of variance plan and observed mean squares for body wt, lung wt, Na^+, K^+, Cl^-, glucose, BUN, cholesterol

SV	df	Body wt	Lung wt	Na^+	K^+	Cl^-	Glucose	BUN	Cholesterol
		Mean squares							
Treatment	1	0.21	316.40	5.06	0.20	0.06	5.06	121.00**	95.06
Period	1	8.73	3.85	33.06	0.01	0.56	280.56	20.25	10.06
Treatment x period	1	67.08	0.19	22.56	0.36	1.56	150.06	12.25	76.56
Error	12	18.15	36.69	8.19	0.10	3.40	92.94	2.96	35.31
Total	15								

**Statistical significance is at P<0.01.

Table B14. Analysis of variance plan and observed mean squares for the radioactivity of ^{14}C in blood, lung tissue, sediment

| | | Mean squares | | |
SV	df	Blood	Lung tissue	Sediment
Treatment	1	18.06	286760.25*	286225.00
Period	1	69.03	11250.00	2450.00
Treatment x period	1	3.78	6670.13	61425.13
Error	12	2673.77	10295.71	149434.04
Total	15			

*Statistical significance is at P<0.05.