THE PATHOLOGY OF SILO GAS TOXICOSIS IN PIGS

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ABSTRACT

THE PATHOLOGY OF SILO GAS

TOXICOSIS IN PIGS

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Research was conducted to determine the composition of silo gas, the effects of silo gas in pigs, and the similarity between the lesions of silo gas toxicosis in pigs, nitrogen oxides toxicosis in rats, and silo filler's disease in man.

The analysis of gas was made with the mass spectrometer. The concentrations in 2 silos were: (a) carbon dioxide, 0.173 to 9.054%, (b) nitric oxide, 0 to 140 parts per million, and (c) nitrogen dioxide, 8 to 360 parts per million.

Pigs were exposed for 3, 4, 5, and 10 minutes to silo gas. All pigs exposed for 10 minutes died within 21 to 72 minutes. All other pigs survived exposure, with the exception of 1 pig exposed for 4 minutes, which died in 10 hours and 27 minutes. Survivors were killed and examined at 15, 29, 30, 60, and 61 days.

In pigs dying from exposure to silo gas, the earliest lesions noted were alveolar edema and hyperemia. The edema was eosinophilic and formed large quantities of fibrin in pigs that survived the longest. In addition, there was hemorrhage, bronchiolar and bronchial epithelial desquamation, and perivascular, interlobular, and subpleural edema. These lesions were similar to those produced in rats by exposure to high concentrations of nitrogen oxides. Pigs surviving the acute effects of

silo gas toxicosis did not develop bronchiolitis fibrosa obliterans, which has been described for silo filler's disease in man.

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To Huda

who made it worthwhile

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INTRODUCTION

> "At first glance the lungs may seem uncomplicated, but many wise men have gone astray in their labyrinths. ... Disease commonly results in a profound but variable revision of their architecture. With each breath, also, the innermost recesses of the respiratory tract are brought very much into contact with a sometimes hostile environment. That we are not more often disabled we owe to their marvelous capacity to recover from injury and to their large reserve."
>
> Averill A. Liebow (1962)

In our diverse and ever-changing environment, new stresses constantly appear. One of the most ubiquitous of these is the nitrogen oxides. As by-products of the combustion of gasoline and of tobacco, they are present in small quantities as pollutants in the air of populated areas of the world. In much higher concentrations, they are also a formidable occupational hazard in certain industrial, military, and agricultural occupations.

In the toxicoses that occur following exposure to concentrations of nitrogen oxides, there is a wide spectrum of lesions. In man one of the most interesting lesions is bronchiolitis fibrosa obliterans (BFO). Although it is a common lesion in silo filler's disease and in other forms of nitrogen oxides toxicosis, it has never been convincingly reproduced in animals by exposing them to pure gaseous nitrogen dioxide. Stokinger (1964) has advanced 2 theories to explain this enigma:

1. Nitrogen dioxide may not be the only toxic gas produced in silos.

2. Animals may respond differently from humans to nitrogen dioxide and, in fact, do not produce BFO as a response to this gas.

This research was an attempt to determine more fully the pathology of silo filler's disease and of BFO. Two things were unique in this study:

1. Exposures were to naturally produced silo gas in freshly filled farm silos, thus simulating the conditions of human exposure.

2. The pig was selected for exposure to silo gas. This animal resembles the human in many aspects of pulmonary structure (McLaughlin et al., 1961) and it has not been used in any of the previous research on nitrogen oxides toxicosis.

REVIEW OF THE LITERATURE

Von Oettingen (1941) published a thorough review of the early literature of nitrogen oxides toxicosis. More recent reviews have been compiled by Gray (1959) and Stokinger (1964). This review, while attempting to give an over-all summary of the many ramifications of nitrogen oxides toxicosis, will be concerned particularly with the literature relating to the disease in man called silo filler's disease.

Chemistry of the Nitrogen Oxides

Stokinger (1964) listed the nitrogen oxides that may contaminate the atmosphere as:

nitrous oxide.....N_2O
nitric oxide.....NO
nitrogen dioxide.....NO_2
nitrogen trioxide.....N_2O_3
nitrogen tetroxide.....N_2O_4
nitrogen pentoxide.....N_2O_5

Grayson (1957) reported that nitrous oxide is commonly used in inducing anesthesia and has no toxic effects, that nitrogen trioxide dissociates very rapidly into nitric oxide and nitrogen dioxide, and that nitrogen pentoxide reacts with air to form nitrogen dioxide and nitrogen tetroxide. Thus, the principal nitrogen oxides from a toxicologic point of view are nitric oxide, nitrogen dioxide, and nitrogen tetroxide.

Nitric oxide is produced in some reactions involving low concentrations of nitric acid (Jolly, 1964; Thienes and Haley, 1964) and in welding operations (Elkins, 1946). Von Oettingen (1941), Grayson (1957), and Thienes and Haley (1964) stated that nitric oxide is quickly

converted to nitrogen dioxide in the presence of air, whereas Elkins (1946) and Stokinger (1964) stated that conversion to nitrogen dioxide is dependent upon concentration of nitric oxide. Elkins (1946) described the kinetics of this conversion and stated that a concentration of 100 parts per million (p.p.m.) nitric oxide is converted to nitrogen dioxide at the rate of 2.8 p.p.m. per minute. This allows high concentrations of nitric oxide to persist long enough to exert toxic effects independently of nitrogen dioxide, and it may account for some of the variability of results in the literature. Pflesser (1936, cited by von Oettingen, 1941) reported that nitric oxide has no irritant properties, but it can induce a severe methemoglobinemia, which may cause death.

Von Oettingen (1941) mentioned that nitrogen dioxide is the most important oxide of nitrogen from the toxicologic point of view. It exists in equilibrium with its dimer, nitrogen tetroxide. Thienes and Haley (1964) reported that at 22 C all the gas is present as nitrogen tetroxide and at 140 C it is present as nitrogen dioxide. Von Oettingen (1941) stated that in whatever molecular form nitrogen dioxide is inhaled it is at once altered to that equilibrium prevalent at body temperature, which is a mixture of approximately 30% nitrogen dioxide and 70% nitrogen tetroxide. Yost and Russell (1944, cited by Rounds and Bils, 1965) stated that the gas, which would reach the lung cells through a film of aqueous tissue fluid, combines with water in the fluid to form nitric and nitrous acids.

Thienes and Haley (1964) stated that nitrogen dioxide is dark brown in color, while nitrogen tetroxide is yellow. The mixture of these gases may be any shade of brown, orange, or yellow. Because nitric oxide, nitrogen dioxide, and nitrogen tetroxide are all in equilibrium

with each other and because they are difficult to separate for purposes of measurement, they are usually collectively referred to as the oxides of nitrogen, nitrogen oxides, or simply as nitrogen dioxide. In this thesis the term nitrogen oxides is used to describe this mixture of gases.

The most commonly used method for the measurement of the nitrogen oxides employs a specific reagent which changes color in their presence. This reagent, a mixture of sulfanilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride, and acetic acid, was first described by Saltzman (1954) and is referred to as the Saltzman reagent. The color change is measured in a spectrophotometer and calibrated with known concentrations of nitrogen dioxide. Gill (1960) compared this method with other earlier methods and described a simplified method for standardization.

Peterson et al. (1958) used the mass spectrometer to measure the nitrogen oxides. They used masses of 30 and 46 to measure nitric oxide and nitrogen dioxide, respectively.

Sources of the Nitrogen Oxides and Their Effects in Man

The most ubiquitous source of the nitrogen oxides is the high temperature combustion of liquid fuels in gasoline motors and diesel engines. Hence, the chemical smogs today plaguing so many American cities are related directly to the density of their motor traffic. Stokinger (1964) has estimated that 200 to 300 tons of nitrogen oxides are emitted daily into the Los Angeles atmosphere, and this city has reported (Mills, 1962) levels of over 3 p.p.m. This approaches the Maximum Allowable Concentration of 5 p.p.m. (Threshold Limit Values for 1960).

Haagen-Smit et al. (1959) found concentrations of 145 to 655 p.p.m. nitrogen oxides in cigarette smoke and over 1100 p.p.m. in pipe and cigar

smoke. Stokinger (1964) confirmed these findings. These levels of nitrogen oxides in the atmosphere would be rapidly fatal to man. There is no evidence to indicate why the same levels in tobacco smoke are so innocuous.

The nonagricultural sources of lethal concentrations of nitrogen oxides are usually related to the use of nitric acid, the detonation of explosives, the operation of carbon and electric arcs and acetylene torches, the incomplete combustion of celluloid and nitrocellulose films, and the combustion of rocket fuels.

Exposures associated with nitric acid have been reported by Wood (1912), Rossano (1945), Adley (1946), Feil (1951), McAdams and Krop (1955), McAdams (1955), and Darke and Warrack (1958). In all these exposures nitric acid came into contact with some substance which it oxidized, yielding large amounts of nitrogen oxides. Cases reported by Wood (1912), Rossano (1945), and Adley (1946) terminated fatally in 26 hours to 10 days after exposure (AE) from pulmonary edema and/or bronchopneumonia. McAdams (1955) and Darke and Warrack (1958) described cases in man that terminated fatally 14 and 27 days AE with bronchiolitis fibrosa obliterans (BFO). Schultz-Brauns (1930, cited by von Oettingen, 1941) has reviewed other fatal cases associated with nitric acid.

Becklake *et al*. (1957) studied the long-term effects of 7 patients exposed for 5 to 75 minutes to blasting fumes from "ammonium dynamite". High concentrations of nitrogen dioxide were believed to be present in the fumes, and pulmonary function studies on the patients for periods up to 64 months after exposure revealed changes consistent with fibrosis of the bronchi and bronchioli and narrowing of their lumina in 2 of the patients. They interpreted these findings as indicative of BFO.

Pflesser (1936, cited by von Oettingen, 1941) and Charleroy (1945, cited by Kooiker et al., 1963) have reported fatalities resulting from explosions on naval vessels in which large quantities of nitrous fumes were produced.

Williman (1935) and Lindqvist (1944) reported 5 deaths from 1 to 4 days AE to fumes produced by electric and acetylene welding in confined spaces. Coltman and MacPherson (1938) and Elkins (1946) described the high levels of nitrogen oxides that may be produced by these procedures. In several nonfatal cases which Lindqvist reported, confluent areas of increased density with a mottling effect that resembled miliary tuberculosis were seen on radiographic examination.

Large quantities of nitrogen oxides may be produced by the slow combustion of celluloid and nitrocellulose film. The Cleveland Clinic disaster (Editorial, J.A.M.A., 1929), in which roentgen film of nitrocellulose type caught fire, resulted in the deaths of 125 people and the hospitalization of 80 to 100 others. Nichols (1930) described 3 of these fatalities. One patient died from pulmonary edema shortly after exposure; another died several days AE from tracheobronchitis, pulmonary edema, and bronchopneumonia; the 3rd died 25 days AE from subacute interstitial pneumonia. Convalescing patients had numerous small nodular opaque areas in the lungs on radiographic examination. These resembled miliary tuberculosis and were diagnosed as exudative infiltration and interstitial fibrosis. The opacities seemed to gradually fade as the patients recovered.

The most recent source of air pollution from nitrogen oxides is the firing of ballistic missiles. Diamond and Johnson (1965) determined the concentration of nitrogen oxides emitted by the test firing of the

Titan II missile and found 29 to 461 p.p.m. at 300 feet and 0 to 32 p.p.m. at 700 feet from the firing point of the first stage. McLouth and Terry (1965) recorded 0 to 80 p.p.m. nitrogen dioxide when air was sampled 300 feet from the exhaust blast of the Titan missile.

Flury (1930, cited by von Oettingen, 1941, and McAdams and Krop, 1955) has distinguished 4 clinical types of nitrogen oxides toxicosis:

1. The irritant gas type is characterized by severe irritation, choking in the throat and chest, and violent coughing, followed by a latent period of several hours when symptoms reside. Following this latent period the patient becomes more dyspneic, cyanotic, and dies 1 to 2 days later from pulmonary edema. McAdams and Krop (1955) stated that this is the phenomenon produced when nitrogen dioxide is the main oxide present.

2. The reversible type is characterized by immediate dyspnea, cyanosis, vomiting, vertigo, loss of consciousness, and methemoglobinemia. This group of patients does not develop pulmonary edema and, if removed from the exposure early enough, may recover completely. Otherwise, the poisoning may rapidly end fatally. According to McAdams and Krop (1955), this type is produced when nitric oxide is the main oxide present.

3. The shock type is characterized by severe symptoms of asphyxiation, convulsions and respiratory arrest, death presumably being due to interference with the pulmonary circulation resulting in stasis in the blood vessels. This form is due to sudden inhalation of very high concentrations of nitrogen oxides.

4. The combined type is characterized by symptoms of central nervous system disorder, such as vertigo, somnolence, and staggering gait. There may be some cyanosis. After apparent recovery, this stage may be followed,

after some hours, by progressive dyspnea, marked cyanosis and pulmonary edema.

Lowry and Schuman (1956b, cited by Stokinger, 1964) reviewed the literature and correlated the clinical and pathologic processes with the concentration of gas. They estimated that acute pulmonary edema, bronchopneumonia, and death in 2 to 10 days usually result from exposure to over 500 p.p.m. nitrogen dioxide. Exposure to 150 to 200 p.p.m. results in fatal BFO in 3 to 5 weeks. Bronchiolitis with focal pneumonitis results from exposure to 50 to 100 p.p.m. Spontaneous recovery usually occurs after 6 to 8 weeks.

Blumgart and MacMahon (1929, Ehrlich and McIntosh (1932), La Due (1941), McAdams (1955), and Spencer (1962) have reviewed the literature and described the clinical and pathologic characteristics of BFO. This lesion was first described by Lange in 1901. Fraenkel (1902, cited by La Due, 1941) was the first to describe it in association with nitrous fume poisoning. Fraenkel divided the clinical course into 3 stages: (1) there was a short period immediately after exposure to the gas in which the patient was dyspneic, cyanotic, and coughing; (2) there was a latent period of variable length during which all symptoms vanished; (3) there was a final period during which symptoms were markedly accentuated, the patient expectorated bloody sputum, coarse rales were heard, and there was increasing emphysema. Lowry and Schuman (1956a) list the causes as (1) inhalation of irritant gases, such as nitrogen dioxide, ammonia, chloropicrin, chlorine, hydrogen chloride, sulfur dioxide, phosgene, and the war gases, and (2) a complication of pneumonia, particularly influenza. Blumgart and MacMahon (1929) have described the pathogenesis of the lesion. Catarrhal bronchiolitis with necrosis and desquamation of bronchial and bronchiolar epithelium are first seen. The inflammatory process involves the basement membrane,

elastic and smooth muscle fibers, and peribronchiolar connective tissue. The bronchioli contain an inflammatory exudate with numerous desquamated epithelial cells, leukocytes, and large quantities of fibrin. Fibroblasts and some capillary endothelial cells proliferate into this exudate and attempt to organize it. The final result is a polypoid mass of fibroblastic granulation tissue, which may or may not be covered by epithelium, projecting into the lumen of the bronchiole. The lumen may be completely obliterated, or there may be a crescentic slit remaining. There is frequently emphysema in the alveoli distal to the obliteration. Grossly these fibroblastic masses appear as firm, irregular nodules 1 to 2 mm. in diameter and scattered throughout the lungs. On radiographic examination they have the appearance of miliary tuberculosis.

The specific lesion of BFO is rare. Lowry and Schuman (1956a) reported that only 2 cases out of a total of 70,281 were diagnosed at the University of Minnesota Medical School between 1919 and 1952.

Experimental Studies of Nitrogen Oxides Toxicosis

The Hygienic Guide Series (cited by McLouth and Terry, 1965) set the odor threshold for nitrogen dioxide as 5 p.p.m. McLouth and Terry reported that odor could be detected when less than 1 p.p.m. was the measured concentration. Because of their findings, the odor threshold at Cape Kennedy is considered to be 1 p.p.m. Henschler *et al.* (1960a, 1960b, cited by Stokinger, 1964) used nitrogen dioxide produced from the action of acid on sodium nitrate and reported that 0.1 p.p.m. could occasionally be detected and 0.4 p.p.m. could always be detected. Stokinger (1964) reported that the threshold of odor perception generally is 1 to 3 p.p.m. for the majority of individuals.

In a series of self-exposures, Lehmann and Hasegawa (1913, cited by von Oettingen, 1941) noted that exposures of 64 p.p.m. nitrogen dioxide caused moderate irritation of the larynx, 100 p.p.m. caused marked irritation, and 207 p.p.m. caused very marked irritation of the nose and larynx, with coughing, increased nasal secretion, and lacrimation. Huie (1962, as reported in personal communication to Stokinger, 1964) exposed volunteers to differing concentrations of nitrogen dioxide. Breathing 50 p.p.m. for 1 minute produced unfavorable reactions in 2 of 7 adult volunteers. About half the volunteers found that brief exposures to 25 p.p.m. were unpleasant but presumably not harmful. The Emergency Tolerance Limits set by the technical manual, Oxides of Nitrogen-Toxicity (1963, cited by McLouth and Terry, 1965) are 35 p.p.m. for 5 minutes, 25 p.p.m. for 15 minutes, 20 p.p.m. for 30 minutes, and 10 p.p.m. for 60 minutes. The Maximum Allowable Concentration (that concentration which can be tolerated 8 hours daily with no harmful effects) set by the Conference of Government and Industrial Hygienists is 5 p.p.m. nitrogen dioxide (Threshold Limit Values for 1962).

Vigdortschick et al. (1937) surveyed 127 men working in industries in which the concentration of nitrogen oxides averaged 2.6 p.p.m. These workers had higher rates of tooth decay, chronic bronchitis, pulmonary emphysema, and tuberculosis than did workers in nitrogen oxide-free environments.

La Towsky et al. (1941), von Oettingen (1941), Gray (1959), Kooiker et al. (1963), and Stokinger (1964) reviewed the early research on the toxicity and pathology of experimental nitrogen oxides toxicosis. La Towsky et al. (1941), Gray et al. (1954a), Comstock and Rue (1961), and Kooiker et al. (1963) described methods for exposing animals to measured concentrations of nitrogen oxides.

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La Towsky et al. (1941) exposed 112 animals (cats, rabbits, mice, guinea pigs, and rats) to concentrations of nitrogen oxides varying from 2.6 to 1000 p.p.m. Continuous exposure to 100 p.p.m. produced death in an average of approximately 5 hours, 400 p.p.m. in 1 hour, 800 p.p.m. in 30 minutes, and 1000 p.p.m. in 19 minutes. In a given concentration, length of survival depended upon length of exposure. Animals survived an average of 21 hours when exposed to 100 p.p.m. for 10 minutes. Methemoglobin was seen frequently in the blood during and after exposure, but it disappeared rapidly when the animal was removed from the exposure chamber. MacQuiddy et al. (1941) reported severe hemoconcentration (due to pulmonary edema), lower blood pressure (due to the effect of nitrite on the smooth muscle of the blood vessels), methemoglobinemia, and simultaneous failure of both heart and respiration in these same animals. Tollman et al. (1941) described the lesions in these animals. Of the fatalities, 6% of those which were exposed to the higher concentration died from immediate asphyxiation due to severe methemoglobinemia. All the vessels were filled with brown blood, and there were no histologic lesions. Death due to pulmonary edema during or several hours after exposure accounted for 88% of the fatalities. Most of these animals died with dyspneic convulsions. Histologically, there was severe pulmonary edema and necrosis of bronchiolar epithelium. Lung weights, expressed as percentage of body weight, increased 2.6 to 4.7 times over controls due to the pulmonary edema. Pneumonitis due to secondary bacterial infection accounted for 6% of the fatalities. These animals died within 1 week after exposure. There were no lesions in the animals that survived the acute and subacute effects of the gas.

Tollman et al. (1941) produced similar lesions in laboratory animals by exposing them to carbon arc fumes. They recorded concentrations of 72 to 140 p.p.m. nitrogen dioxide in the fumes.

Gray et al. (1952) demonstrated that exposure of rats to 9 to 14 p.p.m. nitrogen oxides at 4-hour periods for a total of 40 to 96 hours produced rhinitis, tracheitis, and pneumonitis. In a later report (1954c) no harmful effects were noted after exposure to 4 p.p.m. for 4 hours daily, 5 days per week, for 6 months. They recommended on the basis of these data that the Maximum Allowable Concentration of 25 p.p.m. be lowered to 5 p.p.m. The median lethal concentration for male albino rats was 138 p.p.m. for 30 minutes and 67 p.p.m. for 4 hours (Gray et al., 1954b). Gray and his associates were the first to use pure sources of nitrogen dioxide and noted that nitrogen dioxide was the primary toxic substance in red fuming nitric acid, but that the presence of acid vapors increased the toxicity 25%.

Ripperton and Johnson (1959) exposed rats to 0.5 p.p.m. nitrogen dioxide for 2 to 6 weeks and noted no histologic changes in the lung or liver. The levels of urinary aspartic acid were significantly increased in the exposed rats.

Carson et al. (1962) exposed rats, rabbits, and dogs to concentrations of 10 to 1000 p.p.m. nitrogen dioxide. They reported that lung weight/body weight ratios provided the quickest and most objective indication of nitrogen dioxide exposure. The threshold concentrations for toxic effects in rats were 104 p.p.m. for 5 minutes, 65 p.p.m. for 15 minutes, and 28 p.p.m. for 60 minutes. In animals that survived exposure there were no significant differences when compared with controls.

Kleinerman and Wright (1961) exposed rats, rabbits, and guinea pigs to nitrogen dioxide for 2-hour periods. Exposures to 150 to 200 p.p.m. killed all rats and 1/3 of the rabbits. With exposures of 15 to 75 p.p.m. they all survived. Immediately after exposure they had acute pneumonitis and pulmonary edema. By the 4th day the acute inflammatory changes had subsided and macrophages and epithelial regeneration were present, especially in the terminal bronchioles. Healing was practically complete in 2 weeks.

Freeman and Haydon (1964) reported that rats exposed to 100 p.p.m. nitrogen dioxide began to die within 24 hours from pulmonary edema. Two-thirds of the rats exposed continuously to 50 p.p.m. died in 48 to 68 days. All rats survived 25 p.p.m. for periods up to 157 days, with hyperplasia of epithelial and connective tissue cells of the bronchioles, accumulation of desquamated cells, and partial airway obstruction causing some emphysema.

Buckley and Balchum (1965) studied oxygen consumption rats, lactic dehydrogenase levels, and aldolase levels in guinea pigs exposed to short- and long-term dosage regimens of nitrogen dioxide. The short-term exposures were to 40 p.p.m. at half-hour periods for a total of 4½ hours. There was a 2-hour rest period between exposures. The long-term exposures were to 15 p.p.m. continuously for 10 weeks. They observed significant increases in oxygen consumption values in splenic and renal tissues of animals on short- and long-term exposures. Hepatic tissue values were increased in animals on short-term exposures only, while lung tissue values were not significantly different in experimental and control groups. Aldolase and lactic dehydrogenase activities were increased by both exposure regimens. The relative change in activity varied widely in different organs, indicating that tissues may respond to nitrogen

dioxide through different mechanisms, or that the effects may not be the same in different organs.

Balchum et al. (1965) demonstrated increased levels of antibodies against lung tissue in the serum of guinea pigs inhaling nitrogen dioxide in concentrations of 5 p.p.m. and 15 p.p.m. The antibodies were detected in dilutions of serum greater than 10^6 by agglutinating latex particles coated with normal lung protein.

Wagner et al. (1965) exposed various species of laboratory animals (dogs, rabbits, guinea pigs, rats, hamsters, and mice) daily to 1, 5, and 25 p.p.m. nitrogen dioxide for periods up to 18 months. At no exposure level did body weights, hematologic values, biochemical indices, and histologic observations differ significantly in experimental and control groups. The only effect of nitrogen dioxide exposure was a slightly increased rate of tumorigenesis in the lungs of a pulmonary tumor-susceptible strain of mice.

Pace et al. (1961) found HeLa cells in tissue culture to survive daily 8-hour exposure to 5 to 10 p.p.m. for 8 days, but 30 minutes' exposure to 100 p.p.m. was quite toxic. Serum added to the tissue culture medium had a profound protective influence. Some cells survived a concentration of 8600 p.p.m. Rounds and Bils (1965) treated tissue cultures of pulmonary epithelial cells with sodium nitrite solutions and studied the chemical and morphological changes. Utilizing oxygen consumption values, they reported a partial reversible inhibition in oxidative activity during treatment with sodium nitrite. They demonstrated similar nuclear and mitochondrial changes in alveolar epithelial cells in tissue cultures treated with sodium nitrite and in animals exposed to 15 p.p.m. nitrogen dioxide in the atmosphere.

Interrelationships between the nitrogen oxides and other factors have been studied by several workers. Fairchild et al. (1959) found that compounds bearing the sulfhydryl group were capable of antagonizing the acute toxic effects of nitrogen dioxide. Antagonism was demonstrated with mercaptans, disulfides, and thio-urea derivatives administered by inhalation or by injection. Wagner et al. (1961) demonstrated similar but less dramatic protection with oil mists. They hypothesized that the oil coated the alveoli, producing a film which protected animals against otherwise lethal exposures to nitrogen dioxide. Purvis and Ehrlich (1963) enhanced the virulence of Klebsiella pneumoniae infections in mice by exposing them to 25 p.p.m. nitrogen dioxide for 2 hours.

Gray (1959), Kooiker et al. (1963), Stokinger (1964), and Wagner et al. (1965) have commented on the confusing and conflicting nature of existing data regarding the concentrations, types of exposure, and responses to the nitrogen oxides. Kooiker et al. (1963) stated:

> "The literature reveals inadequate data concerning the hazard of such poisoning. Experimental studies are numerous, but the data are conflicting, and comparison of results is difficult. Few reports include sufficient information to permit evaluation of the validity of the reported findings."

Silo Gas Toxicosis in Man

The literature describing silo gas toxicosis is scattered throughout both medical and agricultural journals. Hayhurst and Scott (1914) recorded the first known deaths due to silo gas. Four men ascended a 40-foot metal silo, which was in the process of being filled. Within 5 minutes all 4 men appeared to be dead. They were removed immediately and were given artificial respiration, but they all died. Lesions observed at necropsy were a dark appearance of the blood, failure of the blood to

clot, cyanosis of the lungs, trachea, bronchi, liver, and kidneys, and capillary injection of the brain. When a lantern was lowered into the silo it went out about 18 inches above the silage. Death occurred in 10 and 42 minutes, respectively, in a guinea pig and a rabbit when they were lowered into the silo. When a dog was placed in the silo it held its nose up in the air and was not affected by the gas. Samples of the gas were found to contain 38% carbon dioxide, 13.5% oxygen, and 48.5% nitrogen, these being the only 3 gases tested for. Attempts were made with animals and with the lantern to detect the presence of poisonous gas in 2 other silos, without avail. It was concluded that the men had become asphyxiated with carbon dioxide.

In 1926, Fabian warned of the dangers from poisonous gases in silo filling in an agricultural bulletin. He reported that large concentrations of carbon dioxide were formed during fermentation and that each year cases of asphyxiation and, in some instances, death occurred.

Le Rossignol (1932), in a letter to the editors of the Journal of the American Medical Association, described a patient with symptoms of coughing, headache, aching joints, and subjective sensations of fever within 4 to 8 hours after working in a silo. Symptoms disappeared within 1 to 2 days. The editors suggested that these symptoms may have been caused by (1) volatile organic acids given off during fermentation, (2) allergic phenomena, (3) a secondary bacterial infection, or (4) a combination of these.

Price et al. (1937) described several fatalities in silos and grain elevators: (1) a 9-year-old child was overcome by the gas present in a pit silo. In an attempt to rescue the child, the mother and a sister were also overcome. All 3 died; (2) a farmer was overcome while trying

to clean out his pit silo. Four of his children attempted to rescue him; all 5 died. (3) Two men entered a silo which had been partially filled the day before; both were overcome and died. (4) Two men were fatally asphyxiated while working in a bin filled with damp corn. (5) A workman was fatally asphyxiated after 15 minutes' exposure in a bin filled with damp barley. (6) A workman was fatally asphyxiated while working in a bin filled with damp oats; another worker went in to rescue him and was also fatally asphyxiated.

Analyses of air samples of 2 of the above bins showed that the air contained 10.2 to 12.65% carbon dioxide. No test was conducted for any other gas.

Three fatalities were reported in a partly-filled silo (Deadly Gases Sometimes Accumulate in Silos, 1939). Other agricultural publications warned of dangers due to high levels of carbon dioxide in the gas from freshly filled silos (Silos, 1939; Briggs et al., 1956, cited by Grayson, 1956).

Credit for detecting the presence and possible hazard of the nitrogen oxides in silo gas must be given to Peterson and his associates (1949), who observed a heavy, yellow-brown gas with a distinct odor on the floor of a silo room. A sample of the gas revealed the equivalent of 151 p.p.m. nitrogen dioxide.

In agricultural bulletins (Peterson et al., 1952; Take Precautions Against Deadly Silo Gases, 1957) farmers were urged to take precautions against nitrogen dioxide poisoning. In 1 study (Peterson, 1958), 5 silos were examined. The gas, visible in 2, was heavy, yellow, irritating, and smelled like bleach. It stained the silage a yellow color, which persisted for days after the gas was gone. An air sample taken near the opening of a pipe draining the silo had 58,500 p.p.m. nitrogen dioxide.

Peterson et al. (1958) studied nitrogen oxides production in artificial silos. They found that nitric oxide was the principle nitrogen oxide produced, that it could occupy up to 14.8% of the air (148,000 p.p.m.), and that the peak point of production was 23 hours after filling. By 60 hours, the concentration of nitric oxide was down to 6% (60,000 p.p.m.). Nitrogen dioxide was present in concentrations up to 10% (100,000 p.p.m.). Ten times as much nitrogen oxides were produced when nitrogen was added as nitrates to the artificial silo, compared to nitrogen added as amino acids. They believed the process for conversion of nitrate to nitrogen oxides to be: nitrate--nitrite--nitrous acid--nitric oxide--nitrogen dioxide. When silage was autoclaved, little or no nitrogen oxides were produced, indicating the importance of bacteria in the formation of the gases.

Most of Peterson's early work appeared in agricultural publications and escaped the early attention of the medical profession. Fostvedt (1951, cited by Grayson, 1956) reported a case of nonfatal asphyxiation attributed to carbon dioxide in a man who entered a silo. Schroeppel (1953), in a letter to the editors of the Journal of the American Medical Association, asked for advice concerning a man found dead in a silo filled 24 hours previously with corn silage. There was a definite slope and the body was found lying in the lowest part of the slope. The editors replied that high levels of carbon dioxide in the silo were probably responsible for the fatality.

The first published study of silo gas toxicosis in which nitrogen dioxide was incriminated was that of Delaney et al. (1956). In their 1st case, a man died 5 days after the beginning of exposure to silo gas. At autopsy, there was extensive congestion and edema of the lungs. In

a 2nd case, a man working in a silo had vague symptoms of constriction in the chest, coughing, and weakness. He remained in bed for a week and then recovered. During this time deaths occurred in some pigs penned near the silo. Two weeks after exposure he became progressively more dyspneic. He was admitted 1 month after exposure with a clinical diagnosis of subacute bronchopneumonia and eventually recovered with supportive therapy. Radiographic examination revealed extensive miliary mottling throughout the lungs, especially in the upper lobes. Delaney and his associates, citing the work of Peterson et al. (1949), attributed the symptoms and lesions in their patients to the possible presence of nitrogen dioxide in the silo gas, and named this new condition silo filler's disease.

Lowry and Schuman (1956) described 4 cases of silo filler's disease. In 2 of their cases 2 men attempted to climb the chute of a silo 1 day after it had been filled with corn silage. Both were exposed to what they described as an irritating, oppressive gas in the chute. They immediately suffered from coughing, dyspnea, and shortness of breath. Their distress never did subside, and they were eventually hospitalized. Radiographic examination revealed scattered focal opacities resembling miliary tuberculosis. They died 27 and 30 days after exposure. Grossly, the lungs contained numerous uniformly distributed lesions which were visible and palpable as firm, discrete nodules of miliary size. Microscopically there was BFO. The bronchioles were filled with a cellular fibrinous exudate, and organization of this adherent plug of fibrin by ingrowth of fibroblasts from the bronchial wall tended eventually to occlude the lumen.

In the other 2 cases there was immediate respiratory distress, then rapid recovery. Latent periods in which no distress was felt were

followed by relapses 10 and 19 days after exposure, with symptoms of progressively increasing respiratory distress and coughing. Radiographic examination revealed the presence of scattered, diffuse, nodular infiltrations. Both patients were treated with prednisolone, which caused a dramatic reversal in symptoms, and they recovered with no ill effects. All 4 cases were diagnosed as BFO resulting from the inhalation of nitrogen dioxide in the silo gas. Lowry and Schuman (1956) stated that the potential concentration of nitrogen dioxide was roughly proportional to the nitrate and nitrite concentration in the silage. Factors known to increase the nitrate concentration were: (1) highly nitrated soil, either naturally occurring or due to heavy nitrate fertilizer application, (2) drought, and (3) immaturity of the plant when ensiled.

Grayson (1956) described 2 cases of silo filler's disease, which he called "nitrogen dioxide pneumonia". In a later article (Grayson, 1957) he defended this term against the more commonly used term, silo filler's disease. In Grayson's 1st case a man entered a silo and was rendered unconscious by a yellow-brown gas present above the silage. He died from fulminating pulmonary edema 29 hours after exposure. In the 2nd case, exposure for 2 to 3 minutes was followed by dyspnea. This patient eventually recovered with no ill effects, but on radiographic examination there were patchy diffuse confluent infiltrations throughout the middle 2/3 of both lungs. The radiologic diagnosis was "chemical pneumonitis".

Dickie (1957, cited by Grayson, 1957) described 2 cases of silo filler's disease, resulting in subacute bronchopneumonia with BFO.

Gailitis et al. (1958) reported a case in which a patient had dyspnea and hyperpnea 2 to 3 weeks after exposure. There were no lesions upon radiographic examination. Treatment with cortisones brought dramatic relief and recovery.

Leib et al. (1958) described a case of chronic pulmonary insufficiency secondary to silo filler's disease. A man was exposed to high concentrations of silo gas which caused burning of the eyes and nose and a choking feeling. He was bedridden for 3 weeks and retained shortness of breath for 1 to 2 years. On radiographic examination there was an accentuation of the bronchovascular markings. On pulmonary function examinations, there was an increase in residual volume, a decrease in the maximum breathing capacity, and a lowered pulmonary compliance value, the latter indicating that there may have been decreased elasticity of the lungs. The signs and lesions were largely irreversible, and were believed to be due to the exposure to silo gas.

Schell (1958) described a similar case in which there was a diffuse, finely nodular infiltration of the lungs on radiographic examination. Treatment for several months with corticosteroids caused symptoms to disappear, but the radiographic lesions did not regress.

Cornelius and Betlach (1960) reported 2 cases of silo filler's disease. In 1 case a man was treated for pneumonia 10 days after exposure to silo gas. He was hospitalized 34 days later with a fine nodulation in the upper 2/3 of both lungs on radiographic examination. Four months after treatment he had no symptoms. His lungs had no lesions on radiographic examination. In a 2nd case a man had severe pulmonary edema after exposure. He improved rapidly after treatment with corticosteroids. There was a patchy infiltration through most of the lungs on radiographic examination, but the lesions were gone 15 days after treatment.

Rafil and Godwin (1961) have written a detailed description of the pathology of BFO in a case of silo filler's disease. A patient admitted with severe dyspnea and hemoptysis had scattered nodular infiltration of

the lungs on radiographic examination. The patient had worked in a silo for 5 hours some 25 days before. He died from cardio-respiratory failure 3 days after admission, despite supportive and corticosteroid therapy. At necropsy the lungs were firm, voluminous, mottled, grayish-red, and equally involved throughout. There were numerous small nodules and hemorrhagic blotches scattered throughout the lung parenchyma. On histopathologic examination there was severe congestion, hemorrhage, edema, fibrin formation, and numerous alveolar macrophages in the alveoli. Bronchiolar walls were edematous and infiltrated with chronic inflammatory cells. The basement membrane was prominent, hyalinized, and thickened. The bronchioles were filled with fibrin and a cellular exudate. In many places this exudate was undergoing organization, the fibrous stroma completely filling the terminal bronchioles and alveolar ducts. There were attempts by the bronchial epithelium to grow over the fibrous protrusions. Some terminal bronchioles, alveolar sacs, and alveoli were completely lined by a hyaline membrane composed of dense fibrinoid material. There was alveolar emphysema near the areas of bronchiolar occlusion.

Silo Gas Toxicosis in Animals

The nitrogen oxides have been suspected in the etiology of pulmonary adenomatosis, a specific disease of cattle. This condition, first described by Monlux et al. (1953, 1955) and Seaton (1957b), is an acute atypical interstitial pneumonia of unknown cause. Seaton (1957a) reproduced the typical lesions seen in field cases by exposing a cow to fumes produced by the action of nitric acid on copper wire. He postulated that nitrogen dioxide was produced in the rumen by the fermentation of forage containing high levels of nitrates and was then eructated and inhaled.

Dougherty et al. (1962) provided a physiologic basis for this by demonstrating that ruminants do inhale part of the gases they eructate. Grayson (1957) reported, on the basis of Seaton's work (1957), that nitrogen dioxide pneumonia does exist in cattle. Seaton was unable (1957a), however, to reproduce the condition by repeatedly pumping the rumen full of pure nitrogen dioxide gas, and Moulton et al. (1963) were unable to find nitrogen oxides on examination of rumen air samples of field cases.

Haynes (1963) described a respiratory condition in cattle which he called "silo filler's disease". The condition was associated with the feeding of corn silage and was characterized by coughing, hyperpnea, and dyspnea. The condition was observed for several months after the silage was processed. A silage sample analyzed for nitrite content was within the normal limits.

Summary

It is evident from this literature review that the nitrogen oxides and silo gas are public health problems. Silo filler's disease is an occupational hazard in farmers and has been recognized and described by a number of workers. Exposures usually occur 1 to 2 days after the silo is filled or partially filled. The farmer is exposed either in the chute or within the silo. The length of exposure may vary from several minutes to several hours. Initially there is respiratory embarrassment and pulmonary edema. Distress may continue throughout the course of the disease, but frequently there is temporary recovery, followed by the return of respiratory distress 2 to 5 weeks after exposure. Death may result from pulmonary edema within 1 to 2 days after exposure or from BPO 2 to 5 weeks after exposure. Recovery is usually complete but may be partial, with permanent debilitating impairment of respiration.

Wagner and his co-workers (1965) made the following comments in their report:

> "Despite all the efforts made in this and past studies, one large question remains. Can nitrogen dioxide per se produce BFO? ... BFO is the common endpoint in many human exposures to silage gases. When generalized fibrosing reactions were found in animals, they occurred when nitrogen dioxide was associated with other contaminants, notably nitric acid vapors. It may be that animals cannot reproduce the human disease from nitrogen dioxide, or it is equally likely that nitrogen dioxide is not the sole exposure in silage gas. Whatever the answer, it would seem that some future research effort should be made to determine the effects of combinations of agents with nitrogen dioxide in animals to attempt simulation of the human response."

MATERIALS AND METHODS

Objectives

The objectives of this research were: (a) to study the pathology of silo gas toxicosis in pigs, (b) to compare the lesions of silo gas toxicosis in pigs with those of reported cases of silo filler's disease in man, (c) to compare the lesions of silo gas toxicosis in pigs with nitrogen oxides toxicosis in rats, and (d) to determine the presence and amounts of the toxic components of silo gas.

Sources, Housing, and Care of Animals

Rats for Experiment 1 came from 2 groups which were raised on a standard laboratory animal diet. Rats 1 through 4 and 1 control weighed an average of 300 Gm. Rats 5 through 9 and the 2nd control weighed an average of 332 Gm. The pig used in Experiment 2 was a 12-week-old Yorkshire male weighing 27.3 kg. and was raised on a conventional grower ration. Pigs for Experiments 3 and 4 were from a Specific Pathogen Free herd in which there was no history of respiratory disease. They were purchased when 46 to 49 days old and were exposed to silo gas when 65 to 69 days old, at which time the average weight was 10.6 kg. Before and after exposures, the pigs were pastured and fed standard rations, free choice. The pigs were given an anthelmintic, piperazine hexahydrate, in their drinking water approximately once a month.

Hematologic and Fecal Examinations

Blood samples were collected periodically before and after exposures. Packed cell volume and hemoglobin (cyanmethemoglobin method) values and

total and differential white cell counts were determined according to methods described by Benjamin (1961). Fecal samples from the herd were collected periodically and examined for parasites according to the flotation method described by Benjamin (1961).

Gross and Microscopic Examination of Tissues

Control rats were killed by intraperitoneal injection of sodium pentobarbital. Pigs not killed by the gas were killed by electrocution. At necropsy of the pigs, bacteriologic examinations were made of lung, kidney, spleen, and intestine using blood agar and MacConkey's agar. Tissues were fixed in 10% buffered formalin. Rat lungs were fixed by ligation of the trachea and slow intratracheal injection of fixative until the lungs expanded to fill the thoracic cavity. The lungs and ligated trachea were removed and placed in a container of fixative until trimmed. Pig lungs were fixed by intratracheal or intrabronchial infusion of fixative at a continuous pressure of 15 to 30 cm. of fixative for 48 to 72 hours, using an apparatus described by Heard (1960). Sections were cut and labeled from the dorsal cranial, middle, and caudal and ventral cranial, middle, and caudal parts of either the right or the left lung from each pig. All tissues were washed and dehydrated in ethyl alcohol, cleared in xylene, and embedded in paraffin. Sections were cut at 7 μ and stained with hematoxylin and eosin for general observations, Gomori's trichrome stain for collagen and smooth muscle, Wilder's reticulum stain for reticular fibers, Mallory's Phosphotungstic-Acid Hematoxylin for fibrin, and Verhoeff's elastic stain for elastin, using procedures described in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D. C. (1957).

Gas Analysis

Gas samples were collected in specially made glass tubes (Figure 1). On 1 side of the tubes #10-30 joints were attached so that they could be mounted to the inlet of the mass spectrometer. Tubes were evacuated with a vacuum pump* for 5 minutes and sealed. Samples were collected in the silos and the exposure chamber by opening 1 of the stopcocks, thus allowing the air to be pushed into the tube. Air samples were analyzed in a mass spectrometer.** All gases with a molecular weight between 12 and 60 were measured. Each sample was analyzed once at room temperature, then cooled with liquid nitrogen, thus liquifying most gases except nitrogen and oxygen, which were then vacuum removed. The sample was allowed to return to room temperature, at which point a 2nd analysis was made. By comparing peak heights in the 2nd sample with that of carbon dioxide (mass 44) which appears in both analyses, a more accurate measurement of the gases in lower concentrations could be made (Figure 2).

Masses 30, 44, and 46 were used to estimate the concentrations of nitric oxide, carbon dioxide, and nitrogen dioxide. Values obtained by this method were compatible with the data of previous reports and with the lesions observed in the animals exposed. Estimates of nitric oxide, nitrogen dioxide, total oxides, and carbon dioxide are listed in TABLE 2. The complete data from all analyses are presented in TABLE 3.

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*Model 1400 Duo-Seal Pump, Welch Company, Chicago, Illinois.

**Model 21-103C, Consolidated Electrodynamics Corporation, Pasadena, California.

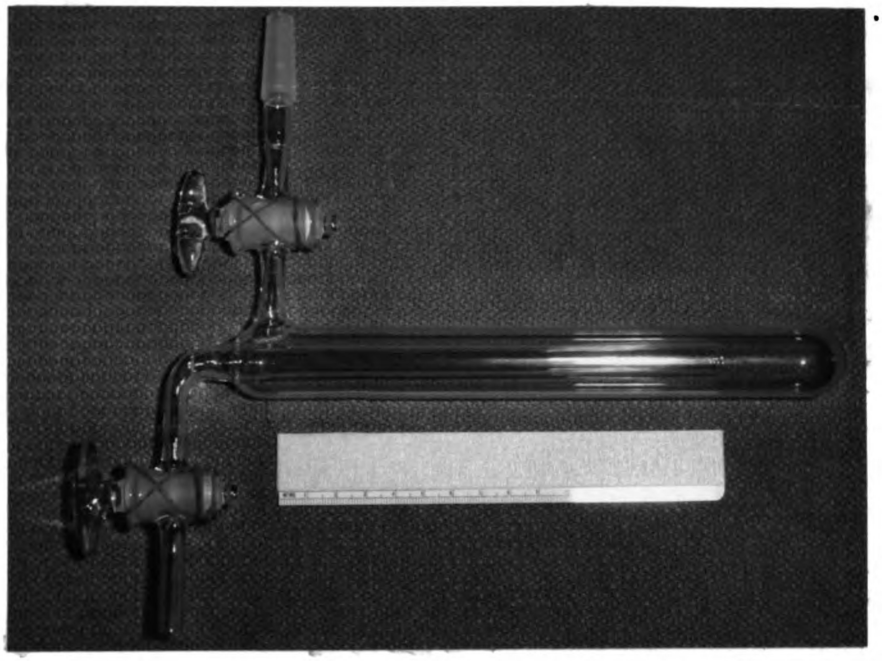

Figure 1. Glass tube used for collecting gas samples.

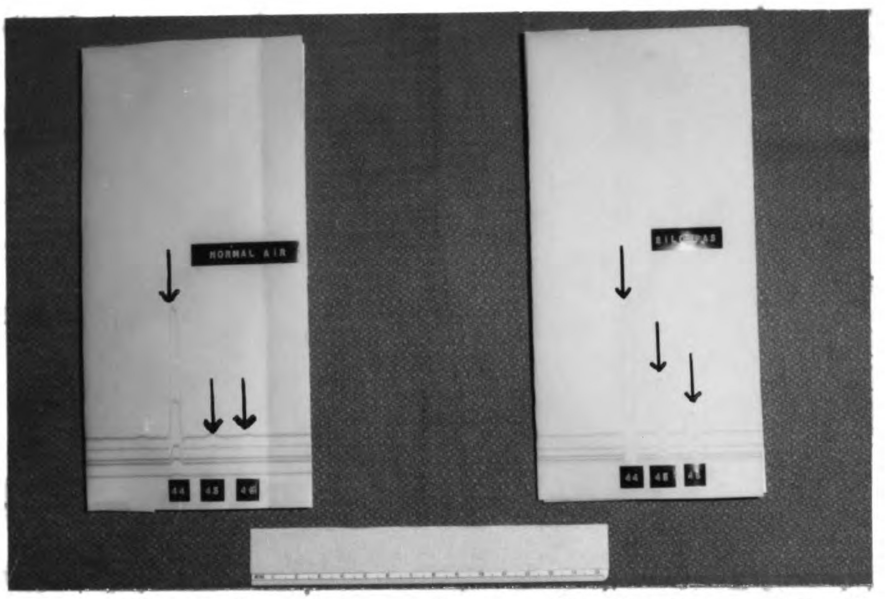

Figure 2. Mass spectrographs showing peaks of masses 44 and 45 (carbon dioxide) and 46 (nitrogen dioxide) in normal air and in silo gas.

General

A rubber mask* covering the face was worn to protect against the inhalation of noxious gases. It was connected to a 50-foot rubber hose, through which inhaled air came from outside the silos. Exhaled air was evacuated directly out through a valve built into the mask.

These experiments were conducted in the fall of 1965.

*Model 4104-P, Davis Emergency Equipment Company, Newark, New Jersey.

EXPERIMENTS AND RESULTS

Experiment 1

Experimental plan. Nine rats were exposed to nitrogen oxides according to the schedule in TABLE 1. Two rats were randomly selected as controls. Exposures were made inside polyethylene plastic chambers measuring approximately 2 x 2 x 4 feet. The rats were caged inside the chamber. Nitrogen oxides were generated by placing measured concentrations of copper wire into a beaker of concentrated nitric acid, using arm-length rubber gloves built into the side of the plastic chamber. The analyses of the gases produced are presented in TABLES 2 and 3. Exposure times were measured beginning at the time the gas reached the caged rats.

Observations and clinical signs. When the copper wire was dropped into the nitric acid, a heavy brown gas poured over the edges of the beaker. The gas was heavier than air and filled the chamber from the bottom up (Figure 3). As soon as it reached the caged rats, they scurried rapidly about in their cages, rubbing their faces frequently with their front paws and gasping. Within 1 minute after the nitrogen oxides had reached Rats 1 through 4, the concentration was so high that the rats were no longer visible. When the cage was removed after 2 minutes of exposure, all the rats were dead.

Rats 5 through 9 were exposed to a lower concentration of nitrogen oxides. They scurried frantically around the cage, often standing on

TABLE 1. Summary of data from exposures of rats to the nitrogen oxides.

Rat No.	MSU Path. No.	Copper wire (Gm.)	Nitric acid (ml.)	Estimated Concentration of Nitrogen Oxides (p.p.m.)	Exposure (min.)	Beginning Exposure to Death (min.)	Histopathology: Alveolar edema	Hyperemia	Fibrin formation	Hemorrhage	Interlobular edema	Epithelial necrosis
1	J 5427	120	300	13,220	2	1-2	+++	+++	+	+	+	0
2	J 5428	120	300	13,220	2	1-2	+++	+++	+	++	++	0
3	J 5429	120	300	13,220	2	1-2	+++	+++	+++	+	+	0
4	J 5430	120	300	13,220	2	1-2	+++	+++	+	+	+	0
5	J 6311	15	200	6,670	12	12	++	++	+	++	+++	+
6	J 6312	15	200	6,670	5	35	++	+	+++	+	+++	+
7	J 6313	15	200	6,670	3	approx. 67	++	+	+++	+	+++	++
8	J 6314	15	200	6,670	3	approx. 94	++	+	+++	+	+++	+
9	J 6315	15	200	6,670	3	approx. 125	+++	+	+++	++	++	+++
controls	J 5426	---	---	---	---	---	0	+	0	0	0	0
	J 6310	---	---	---	---	---	0	0	0	0	0	0

0 = absent or normal + = present ++ = moderate +++ = severe

Figure 3. Caged rat. Nitrogen oxides produced by the action of nitric acid on copper wire.

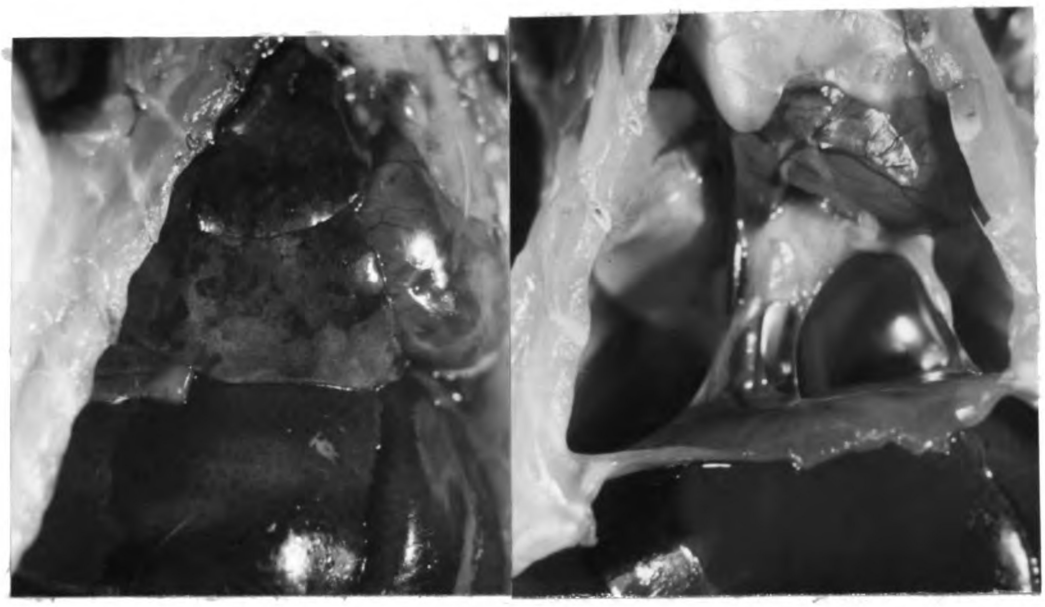

Figure 4. Thoracic cavities of exposed rat (left) and control rat. Dark brown discoloration of tissues, pulmonary edema, and congestion in exposed rat.

their hind legs in order to breathe the less concentrated gas higher off the floor of the chamber. They rubbed their eyes and nostrils frequently. After 2 to 3 minutes of exposure their activity lessened and they became lethargic. Rat 5 died in the chamber after 12 minutes of exposure. The survivors were dyspneic, hyperpneic, and lethargic after removal from the chamber. They gradually became comatose before death. Survival times and histopathology are summarized in TABLE 1.

Gross pathology. The hair and skin of all exposed rats were stained a light yellow color by the gas. The blood and viscera of Rats 1 through 5 were brownish-red. The viscera were congested. The lungs were brown, edematous, hyperemic, and did not collapse (Figure 4). Rat 6 had a slight brownish discoloration of the tissues and blood. Rats 7 through 9 had no discoloration but did have congested viscera and edematous, hyperemic, uncollapsed lungs. There was much blood-tinged froth in the trachea and bronchi.

Histopathology. Rats 1 through 4 were exposed to approximately 13,220 p.p.m. nitrogen oxides (TABLE 2) and died within 1 to 2 minutes after the beginning of exposure with massive pulmonary edema. In some sections alveoli, alveolar ducts, respiratory and terminal bronchioles were filled with an eosinophilic proteinaceous fluid (Figure 6). The edema was more severe near the peripheral alveoli, especially near the ventral surface of the lungs. The lungs were severely hyperemic, and some alveoli contained erythrocytes. In Rats 1 and 4 there was rupture of some bronchiolar arteries with perivascular hemorrhage (Figure 7). Strands of fibrin could be seen in some of the alveoli which were not so edematous. In all exposed rats there was a brownish discoloration of

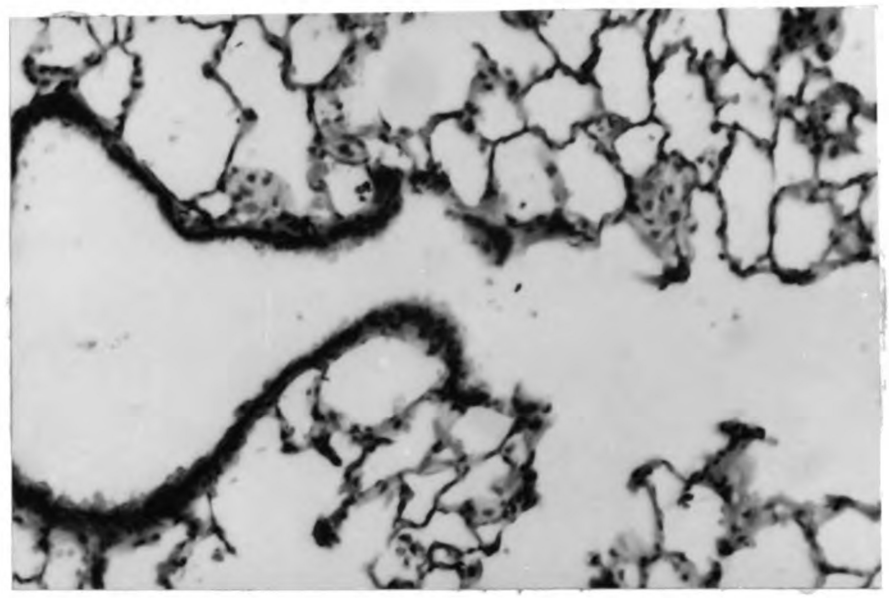

Figure 5. Lung of a control rat. Hematoxylin and eosin. x 188.

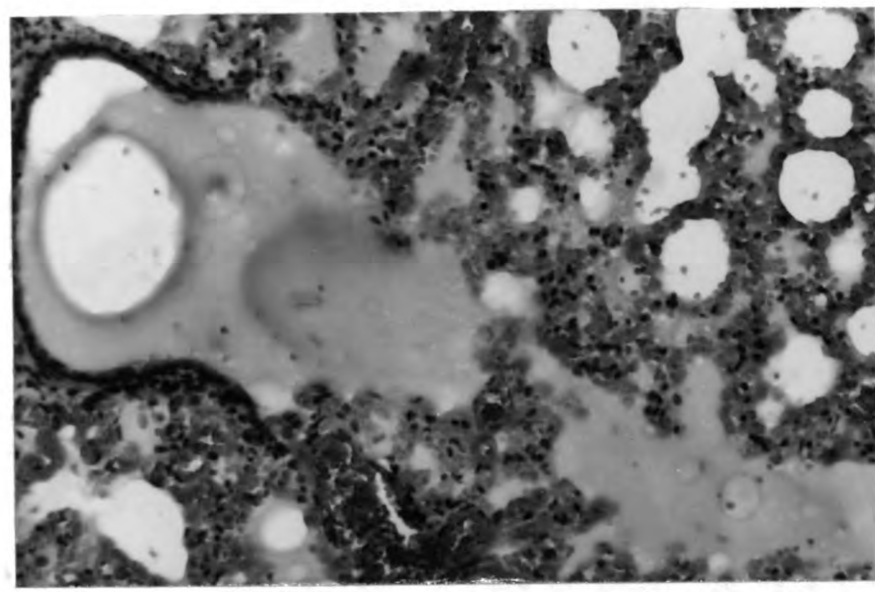

Figure 6. Hyperemia, edema, and hemorrhage of the terminal bronchiole, alveolar ducts, and alveoli. Rat 2, which died 1-2 minutes after the beginning of exposure. Hematoxylin and eosin. x 188.

the erythrocytes in areas surrounding the bronchioles. This was more pronounced in Rats 1 through 4. There was slight peribronchiolar, perivascular, and interlobular edema. There was no evidence of necrosis or desquamation of alveolar, bronchiolar, bronchial, or tracheal epithelium.

Rats 5 through 9 were exposed to approximately 6,670 p.p.m. nitrogen oxides (TABLE 2) and died 12 to 125 minutes after the beginning of exposure. The degrees of alveolar edema and hyperemia generally decreased in comparison to Rats 1 through 4. Fibrin formation in the alveoli, the presence of interlobular, perivascular and peribronchiolar edema, and the degree of epithelial necrosis were increased in comparison to Rats 1 through 4 (Figure 8). All the changes became more pronounced as the rats survived exposure for longer periods of time.

Rats 7, 8 and 9 had extensive fibrin formation in the alveoli. The fibrin strands occurred either singly or in loose bundles (Figures 9 and 10). Epithelial necrosis was manifested, particularly in Rat 9, by sloughing of the bronchiolar epithelium, with the accumulation of almost normal appearing cells in the lumina of the alveolar ducts, bronchioles, and bronchi.

In the kidneys of Rats 5 through 9, and in 1 control rat (these rats were from 1 group), there was increased cellularity and swelling of the glomeruli with adhesions between the parietal and visceral layers of Bowman's capsule. There was dilation of collecting tubules and eosinophilic, homogeneous casts were present in some of them. In Rats 5 through 9, especially in Rats 7 and 8, there was swelling of the convoluted tubules around some glomeruli to such an extent that no lumina were visible. There was marked swelling and congestion of the glomeruli so that no Bowman's space could be seen. These changes appeared to be

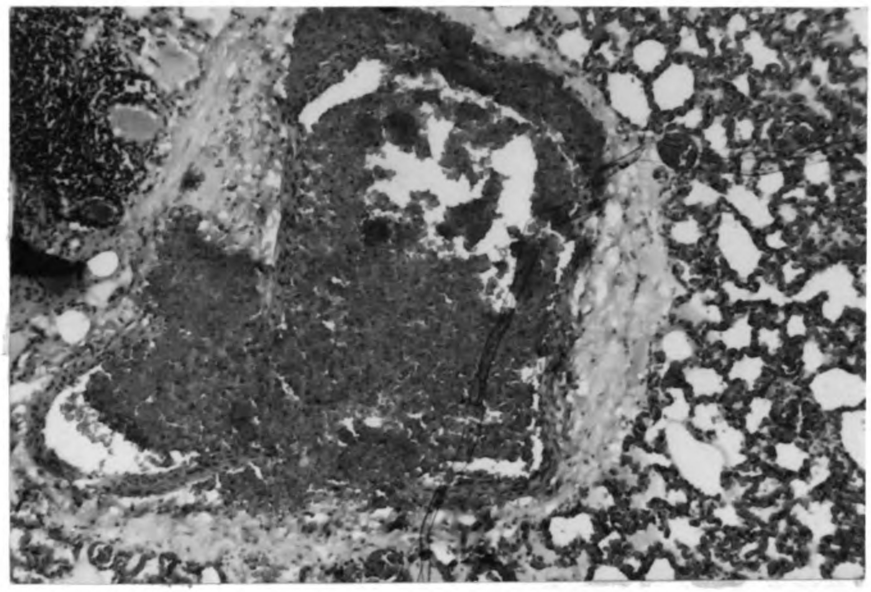

Figure 7. Rupture of bronchiolar arteriole. Rat 1, which died 1-2 minutes after the beginning of exposure. Hematoxylin and eosin. x 75.

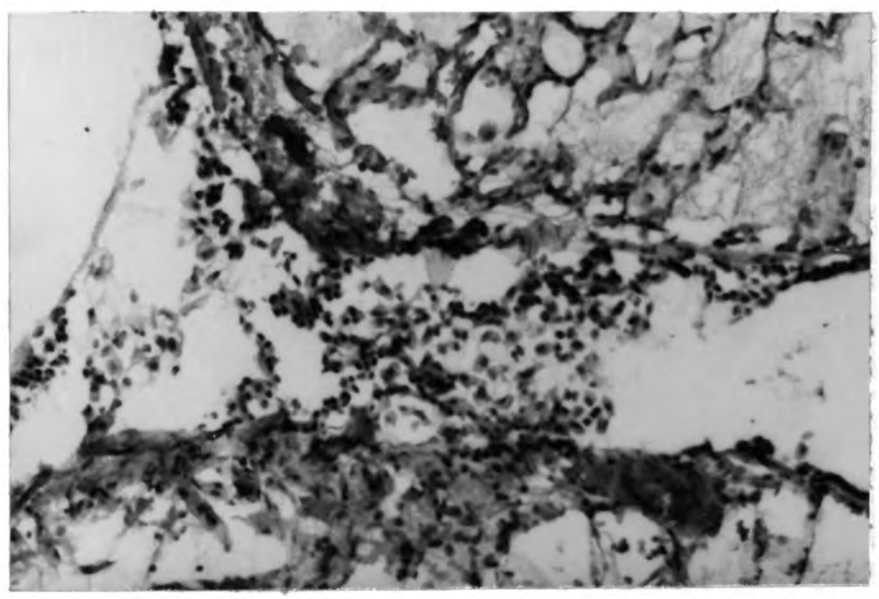

Figure 8. Cellular debris in the bronchiolar lumen. Alveolar macrophages and desquamated bronchiolar epithelium. Rat 9, which died 125 minutes after exposure. Hematoxylin and eosin. x 188.

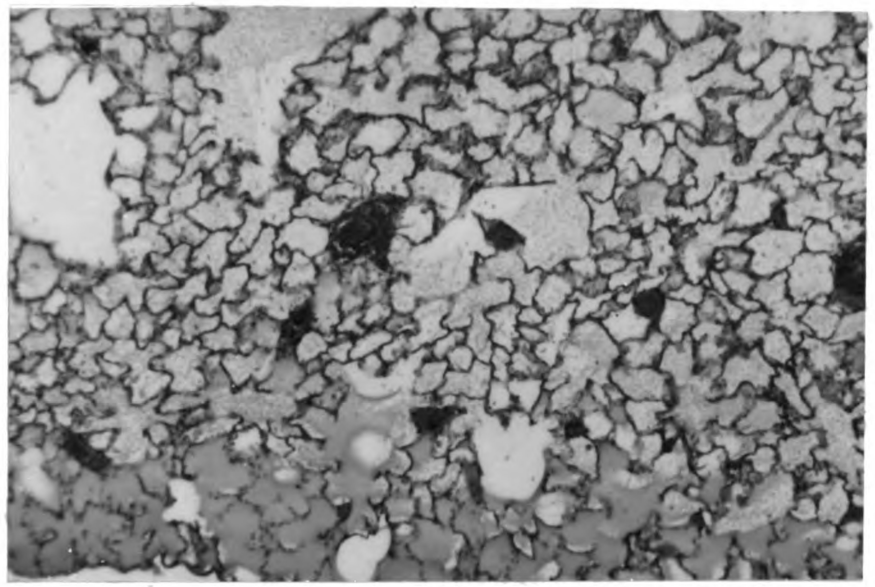

Figure 9. Formation of fibrin from proteinaceous edema. Rat 9, which died 125 minutes after exposure. Hematoxylin and eosin. x 75.

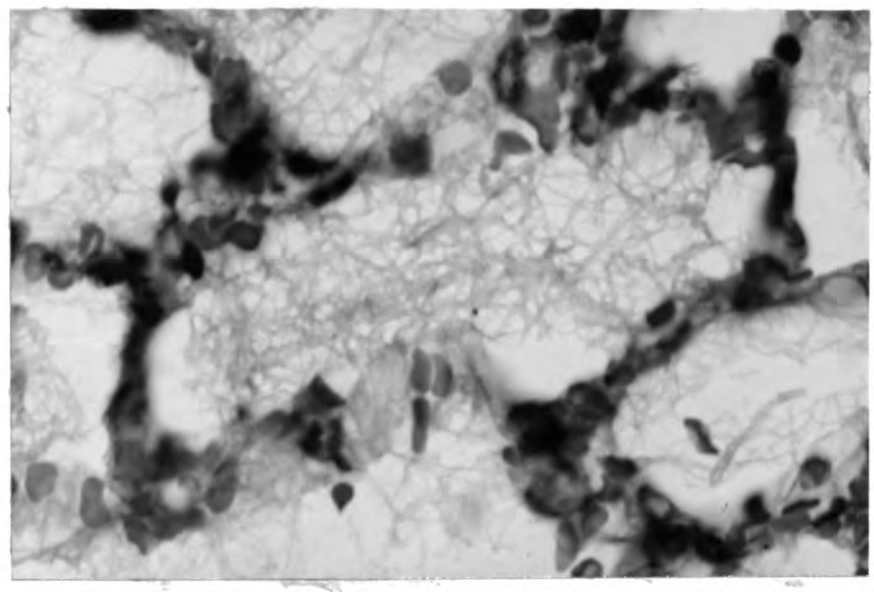

Figure 10. Fibrinous alveolitis. Rat 9, which died 125 minutes after exposure. Hematoxylin and eosin. x 750.

confined to certain areas in the renal cortex. Between and around these swollen areas the renal architecture appeared relatively normal. In all exposed rats there was congestion of the kidneys, liver, spleen, and intestines. Centrolobular congestion of the liver was especially marked in Rats 6 through 9.

Experiment 2

Experimental plan. A concrete silo 50 feet high and 12 feet in diameter was filled approximately 1/8 full of freshly chopped corn silage, so that at its peak it was 12 feet high and at its edges it was 2 to 6 feet high. Approximately 24 hours after filling, a 12-week-old male Yorkshire pig weighing 27.3 kg. was placed in a wire cage located on the edge of the silage. The objectives were to observe the formation of the silo gas and its effect upon the pig.

Observations and clinical signs. Approximately 44 hours after the silage was placed in the silo a heavy, brownish-orange gas was observed. It appeared to extend upwards approximately 30 cm. above the silage at the edge of the peak. The caged pig was moved down into the gas, but the cage was large enough to permit the pig to sit with his head up. In this position the gas came up only halfway to the height of his nose, and after 20 minutes there were no signs of respiratory distress. He was removed from the cage and held in a horizontal position in the gas. Immediately he began to display hyperpnea, dyspnea, and coughing. He was held in this position for 15 minutes, by which time he exhibited open-mouthed breathing. He was then placed back in the cage, where he sat up and breathed with difficulty. Eighty-five minutes later he was dead.

Gross pathology. There was yellow discoloration of the skin due to the silo gas. Some hyperemia of the skin over the abdomen was observed. All the abdominal viscera were congested. The turbinates, trachea, and major bronchi were severely inflamed, and blood-tinged froth was present in the trachea. The lungs were very hyperemic and slightly edematous, particularly in the ventral portions.

Histopathology. There was severe rhinitis and tracheitis with desquamation of epithelium and accumulation of epithelial cells in a fibrinomucous exudate. The desquamated epithelial cells were columnar, and most of them appeared to be morphologically normal. Within the lungs the desquamation of epithelium continued down to the respiratory bronchioles. A cellular exudate consisting of bronchiolar and bronchial epithelium, alveolar macrophages, erythrocytes, and fibrin was found in the bronchi, bronchioles, and occasionally in the alveoli. There was severe general hyperemia, and in the ventral parts of the lung there was extensive alveolar and interlobular edema with fibrin forming in many of the alveoli. Some lobules appeared atelectatic and very hyperemic in comparison to surrounding lobules. The bronchioles in these lobules were lined by intact epithelial cells. Throughout the lungs there were many eosinophils scattered diffusely and in clusters around vessels and bronchioles and in the interlobular connective tissue.

There was congestion of the viscera and the meningeal vessels. There was a focal interlobular eosinophilic hepatitis. This lesion and the eosinophils in the lungs resembled the lesions produced by larval migration of some porcine parasites (Jubb and Kennedy, 1963).

Experiment 3

Experimental plan. A concrete silo measuring 10 x 50 feet was filled 2/3 full of freshly chopped corn silage 36 to 42 hours before exposure. The silo was connected to a dairy barn by a small room which enclosed the bottom of the silo chute. Two plastic sheets were taped in an overlapping fashion to the bottom of the chute so that they hung down to the floor. The sheets were put into place 5 hours before exposure. Twelve pigs were exposed in 4 groups of 3 pigs each for 10, 5, 4, and 3 minutes by lifting up the plastic sheets and pushing the caged pigs under them. Air samples were taken before and after the 10-minute exposure and during the 5- and 4-minute exposures (TABLES 2 and 3). Survivors were killed at 15, 29, 30, 60 and 61 days after exposure. Four litter-mate pigs were killed at 6 hours and at 30, 60, and 61 days after exposure of the experimental pigs to obtain tissues for control data.

Observations and clinical signs. Before exposure it was noted that the spilled silage near the bottom of the chute was stained orange-yellow. An orange haze could be seen in the silo chute. The odor of the nitrogen oxides was strong, and the concentration seemed to increase after the plastic sheets were put into place.

Within 1 to 3 minutes after the beginning of exposure, the pigs began coughing, sneezing, and struggling to get out of the cage. There was hyperpnea, dyspnea, open-mouthed breathing, and ataxia after removal from the gas. Froth dripped from their mouths and nostrils. They died 21, 39, and 72 minutes after the beginning of exposure. The pigs that died at 39 and 72 minutes had convulsions and running movements of the

TABLE 2. Concentrations of carbon dioxide, nitric oxide, nitrogen dioxide (as estimated by peaks 44, 30, and 46 on mass spectrographs) and total nitrogen oxides in the gas samples.

		Experiment 1		Experiment 3				Experiment 4	
	Normal air	Gas produced by action of 300 ml. HNO_3 on 120 g. copper	Gas produced by action of 200 ml. HNO_3 on 15 g. copper	Sample taken before exposure of pigs for 10 min.	After 10 min.	After 5 min.	After 4 min.	Sample taken before exposure	Sample taken after exposure
Carbon dioxide (%)	.40	.362	.423	4.015	5.478	7.660	9.054	.173	.295
Nitric oxide (p.p.m.)	none	8,470	3,620	90	100	140	130	1	none
Nitrogen dioxide (p.p.m.)	none	4,750	3,050	160	210	290	360	8	11
Total nitrogen oxides (p.p.m.)	none	13,220	6,670	250	310	430	490	9	11

TABLE 3. Composition (in percent) of gas samples analyzed (B = before removal of air; A = after removal).

	Normal air		Experiment 1				Experiment 3					
			Gas produced by action of 200 ml. nitric acid on 15 g. copper		Gas produced by action of 300 ml. nitric acid on 120 g. copper		Sample taken before exposure of pigs for 10 minutes		Sample taken after exposure of pigs for 10 minutes		Sample taken after exposure of pigs for 5 minutes	
Mass	B	A	B	A	B	A	B	A	B	A	B	A
12							.434	6.858	.557	6.491	.786	2.428
13								.065		.066		.074
14	8.831	..557	9.068	6.657	9.039	5.072	9.507	.082	8.495	.099	3.147	.112
15				1.133		.579						
16	2.989	4.735	3.022	6.515	2.966	9.420	3.038	7.549	3.064	7.290	3.410	8.071
17	.271	20.891		3.682	.282	2.898	.108	.592	.092	1.498		.149
18	.679	51.810	.120	6.232	.564	8.115	.412	1.940	.278	5.492	.629	.560
19		.835		.141		.144						
20	.271		.120		.141		.151		.139		.157	
22				.141		.144	.086	1.299	.139	1.231	.157	1.382
27								.032				
28	69.293	4.178	70.133	8.215	69.209	3.768	65.769	7.993	65.923	7.689	67.156	8.520
29	.543	.278	.604	.283	.564	.434	.499	.115	.510	.099	.524	.112

TABLE 3--continued

	Normal air		Experiment 1				Experiment 3					
			Gas produced by action of 200 ml. nitric acid on 15 g. copper		Gas produced by action of 300 ml. nitric acid on 120 g. copper		Sample taken before exposure of pigs for 10 minutes		Sample taken after exposure of pigs for 10 minutes		Sample taken after exposure of pigs for 5 minutes	
Mass	B	A	B	A	B	A	B	A	B	A	B	A
30			.362	47.592	.847	47.826		.164		.133		.149
31		.278		.424		.288		.032		.033		
32	15.081	.835	15.114	1.699	14.830	.724	14.781	.098	14.206	.066	14.952	.074
34							.065		.046			
36												
39												
40	1.222		1.209	.141	1.129		1.085		1.021		1.154	
41												
43								.016		.033		
44	.407	14.206	.362	9.065	.423	9.420	4.015	72.039	5.478	68.908	7.660	77.354
45		.278		.141		.288	.043	.822	.046	.599	.104	.710
46				7.648		10.579		.296		.266		.298
47				.283		.288						

TABLE 3--continued

	Experiment 3 (cont.)		Experiment 4			
	Sample taken after exposure of pigs for 4 minutes		Sample taken before exposure		Sample taken after exposure	
Mass	B	A	B	A	B	A
12	.855	6.790	.049	3.792	.049	1.626
13		.073		.049		.024
14	8.783	.073	9.970	.147	9.605	.049
15				.049		
16	3.243	7.667	2.827	5.269	2.807	3.843
17	.225	.511	.124	9.751	.369	17.048
18	.765	1.679	.421	31.519	1.182	55.678
19				.049	.172	.098
20	.180		.173	.073		.123
22	.135	1.350		.738		.320
27				.049		.024
28	61.711	8.105	68.948	5.171	67.980	2.562
29	.450	.109	.496	.147	.492	.049

TABLE 3--continued

	Experiment 3 (cont.)		Experiment 4			
	Sample taken after exposure of pigs for 4 minutes		Sample taken before exposure		Sample taken after exposure	
Mass	B	A	B	A	B	A
30		.109		.024		
31		..036		.147		
32	13.378	.073	15.625	.246	15.812	.147
34	.045		.049	.024	.049	
36						.024
39				.024		
40	1.036		1.140	.024	1.182	
41				.024		
43				.073		
44	9.054	72.289	.173	41.861	.295	18.107
45	.090	.839		.541		.197
46		.292		.196		.073
47						

limbs 1 to 2 minutes before their heart beats were no longer detectable.

The group exposed for 5 minutes had less severe respiratory distress, and their respirations returned to normal in approximately 6 hours. One of the pigs exposed for 4 minutes died 10 hours and 27 minutes after exposure. Signs of respiratory distress persisted for 12 to 18 hours in the other 2, but they survived the effects of exposure. The group exposed for 3 minutes had signs of respiratory distress for only 1 to 2 hours. In pigs dying from the acute effects of the gas, concentrations of nitrogen oxides, survival times, organ weights, and lesions are summarized in TABLE 4.

Gross pathology. Pigs in this group include those which died 21, 39, 72 minutes, and 10 hours and 27 minutes after exposure. There was severe edema and hyperemia in the lungs of all exposed pigs. A progression of lesions could be seen in the pigs surviving the longer periods after exposure. They had more pulmonary hyperemia, hemorrhage, and edema. The hemorrhages were patchy in distribution, 0.5 to 2 cm. in diameter, and involved the superficial and deep lung parenchyma equally. There was froth in the trachea, which became blood tinged and more voluminous in the pigs surviving exposure longer. The nasal passages, turbinates, and trachea were very hyperemic. There was congestion of all the viscera.

In both the exposed and control pigs there were approximately 40 to 60 white spots, 1 to 2 cm. in diameter, scattered diffusely over the liver. They resembled the lesions produced by the larval migrations of Ascaris lumbricoides or Metastrongylus spp. (Jubb and Kennedy, 1964).

TABLE 4. Identification of pigs dying 0-12 hours after exposure to silo gas, survival times, organ weights and histopathologic lesions.

MSU Path. No.	Exposure time (min.)	Estimated concentration of nitrogen oxides (p.p.m.)	Survival time (min.)	Lung weight (Gm.)	Body weight (kg.)	Lung wt. expressed as % of body wt.	Eosinophilia	Alv. edema	Hyperemia	Hemorrhage	Fibrin Forma.	Perivascular & interlobular edema	Epithelial necrosis
058852	10	250-310	21	280	9.09	3.08	++	+	+	+	+	++	+
058831	10	250-310	39	450	12.75	3.53	++	++	++	++	++	++	++
058847	10	250-310	72	430	9.54	4.50	++	++	+++	+++	+++	+++	+++
058842	4	490	10 hr., 27 min.	550	13.67	4.02	+	+++	+++	+++	+++	++	+
058849	control	---	---	255	13.18	1.949	+++	0	0	0	0	0	0

0 = absent
+ = present
++ = moderate
+++ = severe

Histopathology of pigs dying from acute effects of silo gas. In general the lesions in exposed pigs were similar to those seen in rats exposed to the nitrogen oxides. In the lungs of all exposed pigs there was hyperemia, hemorrhage, fibrin formation, and alveolar, perivascular, and interlobular edema. There was an increase in the degree and intensity of these lesions as pigs survived for longer periods following exposure. The edema was more severe in the peripheral alveoli near the pleural surface (Figure 12). Hemorrhage was very severe in the pigs surviving for 72 minutes and for 10 hours and 27 minutes AE, and some of the alveoli were completely filled with erythrocytes. Hemorrhage, edema, and fibrin formation were usually seen together (Figure 13). They were patchy in distribution, and in some sections the entire lobule was involved. In general the dorsal parts of the lungs were slightly more involved than the ventral parts. There were varying degrees of desquamation and necrosis in the epithelial lining cells of the turbinates, trachea, bronchi, and bronchioles of exposed pigs. In the pigs exposed for 10 minutes desquamation and necrosis became more severe as the pigs survived longer periods, and it was most severe in the pig surviving 72 minutes. In the pig which survived 21 minutes AE, spaces appeared between the epithelial cells and their basement membranes, but the cells retained their relationship to each other. In the pigs surviving 39 and 72 minutes AE the cells became more detached from the basement membrane and desquamated in large numbers, both singly and in layers (Figure 14). Desquamated cells, many of which retained their columnar shape and normal morphologic characteristics, filled the lumina of bronchi and bronchioles and were occasionally seen in the alveoli. In the pig exposed for 4 minutes, however, there

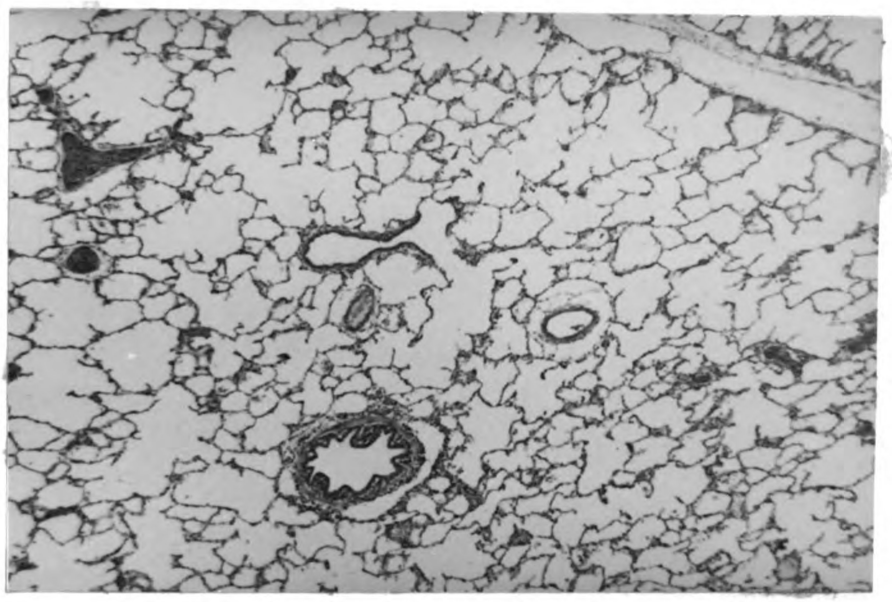

Figure 11. Lung of a control pig. Hematoxylin and eosin. x 62.

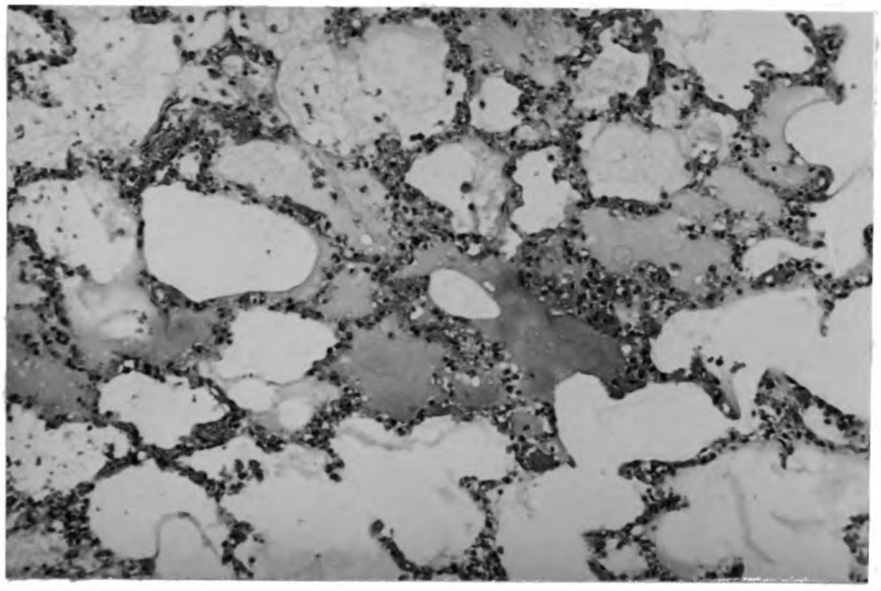

Figure 12. Hyperemia and edema. Pig 058852, which died 21 minutes after exposure. Hematoxylin and eosin. x 150.

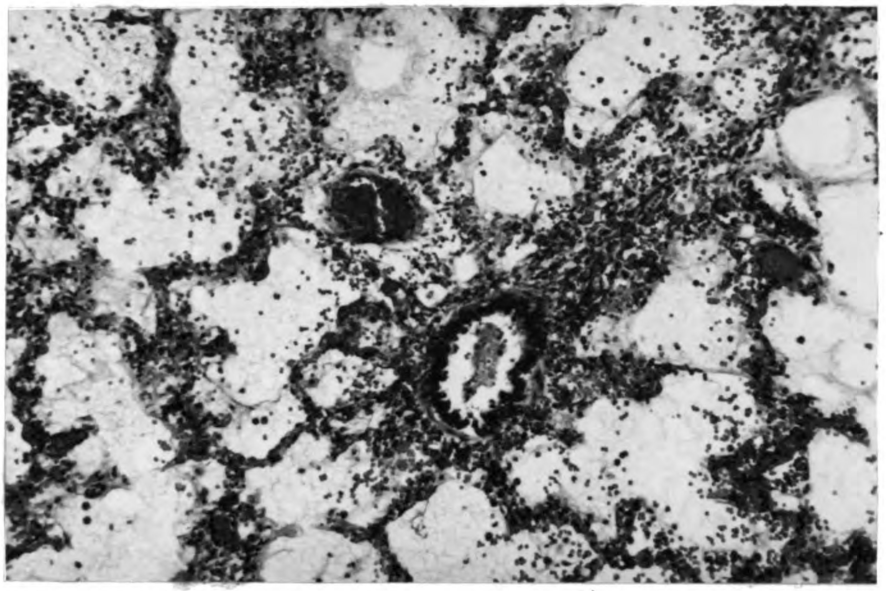

Figure 13. Hemorrhage, edema, and fibrin formation in the alveoli. Pig 058831, which died 39 minutes after exposure. Hematoxylin and eosin. x 150.

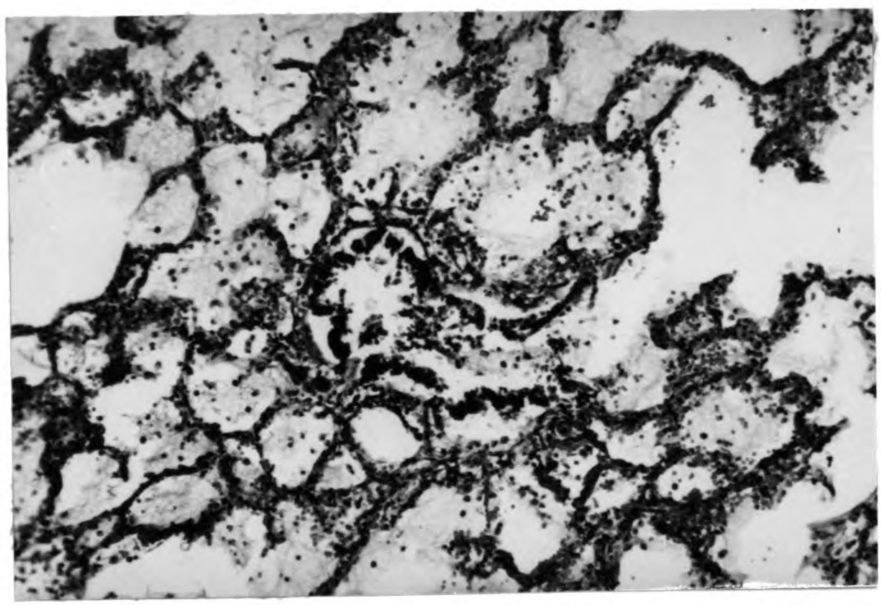

Figure 14. Desquamation of bronchiolar epithelium. Pig 058847, which died 72 minutes after exposure. Hematoxylin and eosin. x 150.

was very little epithelial necrosis or desquamation, even though fibrin formation and hemorrhage were most severe in this pig (Figures 15 and 16). There was congestion of the viscera in all exposed pigs.

There was scattered focal interlobular eosinophilic hepatitis in both exposed and control pigs. There was also a diffuse eosinophilic infiltration in the lungs of both exposed and control pigs. The eosinophils were occasionally clustered together in the alveoli, around bronchioles, and in the interlobular connective tissue. These lesions were attributed to the larval migrations of Ascaris lumbricoides and Metastrongylus spp.

Gross pathology of pigs killed 15 to 61 days AE. In both exposed and control pigs the lungs were normal in color and consistency. There was lobular emphysema in the peripheral parts of the diaphragmatic lobes. This emphysema became more severe and was occasionally observed in conjunction with atelectasis in the pigs killed at 60 to 61 days AE. In the distal portions of the more dorsal bronchi of the diaphragmatic lobe large numbers of nematodes 3 to 5 cm. in length were consistently found. Occasional petechial hemorrhages on the surface of the lung were attributed to electrocution.

The livers of all pigs contained numerous white spots. These were 0.5 to 2 cm. in diameter and numbered from 10 to over 200 in the individual pigs. Roundworms were observed in the middle 3rd of the small intestine of some pigs.

Histopathology of pigs killed 15 to 61 days AE. In both exposed and control pigs there were parasites present in the lungs. These were identified as Metastrongylus spp. on the basis of their size, location in the lungs, and the lesions they produced. There were no differences between

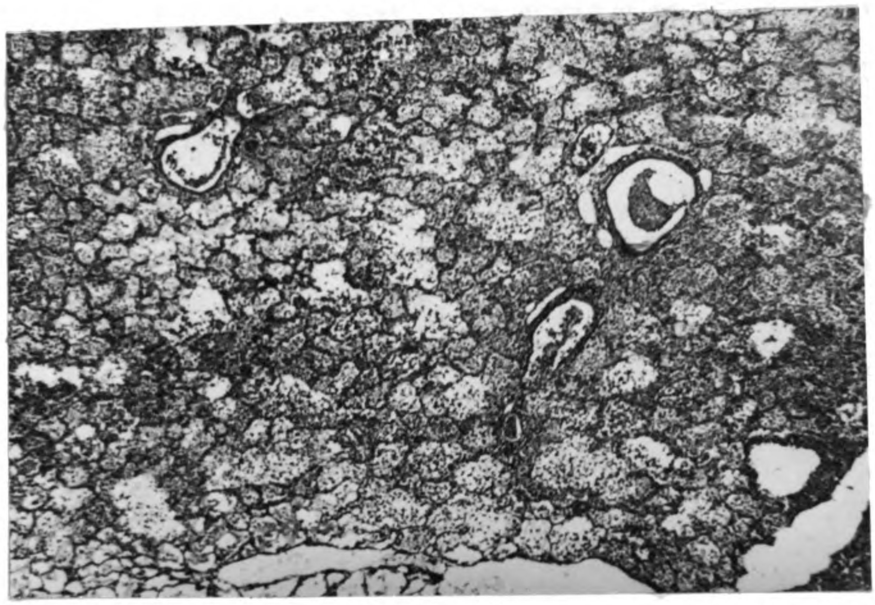

Figure 15. Fibrinous hemorrhagic alveolitis and bronchiolitis. Pig 058842, which died 10 hours and 27 minutes after exposure. Hematoxylin and eosin. x 62.

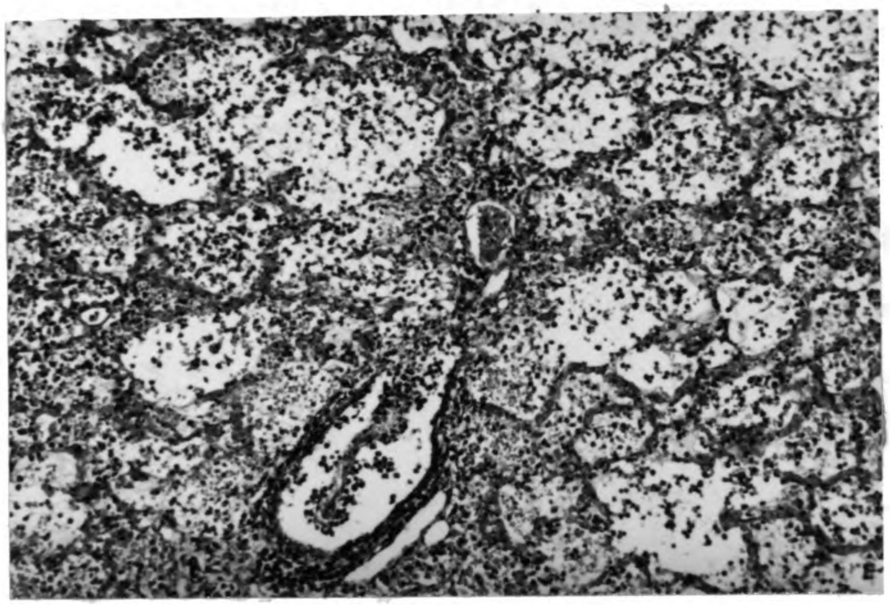

Figure 16. Leukocytes, erythrocytes, and fibrin in the alveoli and bronchioles. Pig 058842, which died 10 hours and 27 minutes after exposure. Hematoxylin and eosin. x 150.

the lungs of control and exposed pigs. The structural alterations in the lung parenchyma caused by these parasites prevented any detailed comparisons. There was no evidence of epithelial necrosis or BFO in any of the pigs.

There were scattered areas of focal eosinophilic hepatitis in the livers of all pigs. These lesions are commonly produced by migrations of the larvae of Metastrongylus spp. and Ascaris spp.

Experiment 4

Experimental plan. A concrete silo 12 x 40 feet was filled to a peak 10 feet high with corn silage cut 40 hours before exposure. Three pigs were placed in a wire cage on the surface of the silage at its lowest point. The exposure was for 11 hours and 10 minutes. Air samples were taken before and after exposure (TABLES 3 and 4). The pigs were killed at 16, 30, and 60 days AE.

Observations and clinical signs. About 4 hours after the silage was placed in the silo, oxides of nitrogen could be smelled. The odor was stronger at 16 hours, but no gas could be seen. At 22 hours there was a brownish discoloration of the silage in the lowest part of the silo, but no gas could be seen, even though the odor was detectable. The pigs were exposed in the silo from 32 to 43 hours after the silage had been put in. They showed no signs of respiratory distress throughout or after the exposure.

Gross and microscopic pathology. These pigs were kept in the same pasture as those on Experiment 3. They also had lesions of pulmonary parasitosis. There were no differences between the tissues in these pigs and the control pigs in Experiment 3.

DISCUSSION

Approximately 15 silos were observed in these experiments. The nitrogen oxides were seen and smelled in 4 silos, smelled in 7 or 8, and neither seen nor smelled in 3 or 4. This research was conducted in the fall of 1965, an unusually dry year. Dry weather is one of the factors which causes plants to concentrate nitrates, thus forming larger quantities of the nitrogen oxides when ensiled (Lowry and Schuman, 1956).

Silo Gas

The nitrogen oxides (some of which are visible) and carbon dioxide (which is not visible) are formed in the silo, gravitate downward through the silage, and (except in silos which are air-tight) leak into the silo chute through the doors in the lower 3rd of the silo. Because of this, the dangers of silo gas toxicosis are equally as great in the chute and at the bottom of the chute as they are inside the silo. The following observations support this belief:

1. Nitrogen oxides were seen and smelled inside silos only when they were less than ½ full and had been allowed to stand for 1 or 2 days without additional silage being added; however, the odor was very strong inside the chutes of most silos observed.

2. Silage which collected on the doors which opened from the chute into the silo was frequently stained brownish-orange by the gas which leaked around the doors into the chute. However, this stained silage was never seen more than 1/3 of the way up the chute. The odor of

nitrogen oxides was frequently quite noticeable at the bottom of the chute, but it could not be detected in the chute at a point above this stained silage and was never detected inside the silo, except in those instances mentioned in part 1 above.

3. Observations in Experiment 2 indicate that an animal's head must be down where the gas collects in order for it to be exposed to the highest concentrations of nitrogen oxides. These observations are substantiated by the report of Hayhurst and Scott (1914). If the gas is visible in the silo, it collects in the lowest part of the surface of the silage and is usually several feet lower than the height of a man's head. It was difficult to expose pigs to toxic concentrations of nitrogen oxides in the silo (Experiments 2 and 4), compared to the ease with which exposures were made in the silo chute in Experiment 3.

In those cases where the gas was seen inside the silo, it appeared 24 to 48 hours after silage was placed in the silo, and it remained visible for less than 12 hours. It tended to appear and disappear earlier on hotter days, probably reflecting the more optimum conditions for microbial fermentation in the silo. The 4 gas samples collected in Experiment 3 (TABLES 3 and 4) show a steady rise in concentrations of carbon dioxide and the nitrogen oxides. They were collected over a 3-hour period and indicate the variation in gas production as time after filling, temperature, and conditions for bacterial growth changed.

Silo gas is a variable mixture of gases. From a toxicologic point of view, they may be divided into:

1. The asphyxiating gases, carbon dioxide and nitric oxide. Concentrations of carbon dioxide found in silo gas in these experiments were estimated to range from 0.173 to 9.054%. Concentrations of nitric oxide were estimated to vary from 0 to 130 p.p.m. (TABLES 2 and 3).

2. The irritant gases, nitrogen dioxide and its dimer, nitrogen tetroxide. Concentrations of nitrogen dioxide in silo gas in these experiments were estimated to vary from 8 to 360 p.p.m. (TABLES 2 and 3).

Prior to the articles written in 1956 (Delaney et al., 1956; Lowry and Schuman, 1956; Grayson, 1956), all deaths and most clinical disturbances caused by exposure to silo gas were attributed to carbon dioxide. Cases described during and after 1956 were attributed to nitrogen dioxide. While descriptions of the history, clinical signs, and lesions were inadequate in many of the early reports, and it may be assumed that many, if not most, of these cases were actually caused by exposure to nitrogen dioxide, it would seem well to keep in mind the asphyxiating gases as causes of toxicosis. The deaths described by Hayhurst and Scott (1914), in particular, seem to have been caused primarily by asphyxiation.

Silo gas toxicosis may be a problem in livestock. In the barn in Experiment 3, the silo room opened into a milking barn with stanchions for cattle. The smell of nitrogen oxides was quite strong throughout the entire milking barn. If cattle had been stanchioned over night with poor ventilation in the silo room and milking barn, they could have been killed by the gas. It has been reported that pigs (Delaney et al., 1956), chickens and cattle (Peterson et al., 1949, 1958) were killed when penned near the bottom of freshly filled silos. In each case, the odor of an irritating gas was reported.

Pathology

The lesions produced in pigs by fatal exposures to silo gas were similar to those produced in rats by exposure to the nitrogen oxides and

varied with the length of survival, which was dependent upon concentration of noxious gases and length of exposure. The first changes produced were hyperemia and alveolar edema. The edema was eosinophilic and protein rich, forming large quantities of fibrin in single strands and in loose clumps in the alveoli and bronchioles if pigs survived longer periods. Hemorrhage and perivascular, interlobular, and subpleural edema were slight in pigs dying soon after exposure and became more severe as they survived longer periods. Desquamation of bronchial and bronchiolar epithelium was seen in all pigs exposed for 10 minutes to the silo gas, becoming more severe as pigs survived for longer periods. Because of these observations, the degree of desquamation is believed due to 2 factors: (1) the amount of nitric and nitrous acids formed in the bronchioles (a factor of the concentration of nitrogen oxides and the length of exposure), and (2) the length of survival of the pig, provided there is sufficient prior insult to kill the epithelium. Longer survival allows the air, rushing in and out of the bronchioles and alveoli, to exert more physical action on the damaged and dying epithelium, causing it to desquamate.

The pig dying 10 hours and 27 minutes AE for 4 minutes to the silo gas had the most severe degree of alveolar hemorrhage, edema, and fibrin formation. Epithelial desquamation was very slight in comparison to the other pigs exposed. There was no evidence of epithelial necrosis or regeneration in the 2 other pigs exposed for 4 minutes, which were killed at 30 and 60 days AE.

Stokinger's 1st hypothesis, that nitrogen dioxide may not be the only toxic gas in silos, is apparently based on the fact that BFO has not been produced in animals exposed to pure nitrogen dioxide, while it does occur in human exposures to silo gas. The following points seem

applicable to Stokinger's statement:

1. Nitrogen dioxide is not the only toxic gas in silos, but it and its dimer, nitrogen tetroxide, are the only irritating gases in silos reported in the literature; and an irritating gas is the only kind known to produce BFO (Lowry and Schuman, 1956).

2. Nitrogen oxides toxicosis unrelated to silo gases does produce BFO in humans (Fraenkel, 1902; McAdams, 1955; Darke and Warrack, 1958) but not in animals (Tollman et al., 1941a, 1941b; Gray et al., 1952, 1954b, 1954c; Ripperton and Johnson, 1959; Carson et al., 1962; Kleinerman and Wright, 1961; Freeman and Haydon, 1964; Wagner et al., 1965). There is no reason to assume that the nitrogen oxides in silo gas should require any additional substance in order to produce BFO in man.

3. In these studies, the 1st in which animals were exposed directly to silo gas and the gross and microscopic lesions reported, BFO did not occur. The toxicity of the silo gas was definitely associated with the odor and appearance of the nitrogen oxides, and it could not be demonstrated when they were not present.

The author believes that nitrogen dioxide and its dimer, nitrogen tetroxide, are the only gases produced in silos that will cause BFO. The 2nd hypothesis of Stokinger seems more plausible: animals may respond differently from humans to nitrogen dioxide and, in fact, cannot produce BFO as a response to this gas.

Necrosis of epithelium is the 1st step in the development of BFO (Blumgart and McMahon, 1929). Yet, it seems apparent that in single concentrated exposures, death in animals (Tollman et al., 1941) is due to asphyxiation or to alveolar edema. Those animals that survive the acute phase of the toxicosis (and any secondary bacterial complications) do

not develop BFO, perhaps because an exposure low enough to permit survival does not cause necrosis of bronchiolar and bronchial epithelium. If this hypothesis is true, it raises the question of why humans do not develop fatal pulmonary edema from exposures concentrated enough to kill bronchiolar epithelium and cause BFO several weeks after exposure or, conversely, why animals are so much more susceptible to the edematigenous action than to the epithelial necrotizing action of the nitrogen oxides.

There are several possible theories to explain this difference in response:

1. The human alveolus could be more resistant to the edematigenous action of the nitrogen oxides, thus permitting the human to survive the high concentrations of gas necessary to produce bronchiolar epithelial necrosis. This might possibly be due to some agent lining the alveoli. Fairchild et al. (1959) described the antagonistic action of sulfhydryl compounds against the acute toxic effects of nitrogen dioxide. Wagner et al. (1961) described the similar but less dramatic protection with oil mists.

2. The bronchiolar epithelium of the human might be more susceptible to injury than that of the animals. Hayek (1962) described the difference in staining quality of mucus in the human bronchiole compared with that of the bronchus. Perhaps this point in the tracheobronchial tree is less protected from irritation.

3. The bronchiolar smooth muscle of the human may constrict the lumen during exposure, lessening the action of the gas upon the alveoli. Many patients exposed to silo gas have complained of pains or choking feelings in their thorax. Some have been exposed without developing immediate dyspnea and hyperpnea, these symptoms appearing several hours

after exposure. The effect on pigs, on the other hand, was immediate and produced dyspnea and hyperpnea within 3 to 4 minutes after the beginning of exposure.

4. Perhaps exposure to an irritant gas produces a fibrinous cellular bronchiolitis in both man and animals, but this exudate is liquified and absorbed in animals while it is organized by fibroblasts and endothelial cells in man. This theory, advanced by Ehrlich and McIntosh (1932), would explain the development of BFO, but it leaves the reasons for the difference in response unanswered.

5. The role of posture may be significant. All animals exposed to nitrogen oxides in the literature had an essentially horizontal tracheobronchial tree. Man, on the other hand, has a vertical tracheobronchial tree. Perhaps the force of gravity, combined with the movements of air and tissue during respiration, has the effect of concentrating inhaled material in the terminal and respiratory bronchioles. The connective tissue lesions of BFO are nodular, not tubular, indicating that there may be a focal necrosis with resulting fibroblastic proliferation. Significant in this respect are the 2 cases reported by McAdams and Krop (1955), caused by an accident in a chemistry laboratory involving nitric acid. One man was showered by the acid, developed severe acid burns of the face, and inhaled large quantities of the nitrogen oxides produced by the acid. He developed severe dyspnea, pulmonary edema, and cyanosis, with secondary emphysema. After several weeks in the hospital he recovered. The 2nd case involved a man in the same laboratory who ran to the aid of the man just described. He assisted him in washing off the acid and administered first aid. This man developed a cough and tightness in his chest for several days after the incident, took sick leave, but never was hospitalized.

His cough continued for 21 days AE, then increased in severity. He was finally hospitalized and died 27 days AE with lesions of BFO. In these 2 cases the heavily exposed man who developed very severe dyspnea and edema and was hospitalized survived. The less heavily exposed man, who was not hospitalized, died from BFO. Perhaps the fact that the first man was hospitalized, presumably in a horizontal position, soon after exposure may have spared him the focal necrotic action of the acids formed by breathing the nitrogen oxides.

It appears that much work is still needed to answer the question of why animals seem to respond to irritating gases without producing BFO. The experiments outlined in this thesis should be repeated, with precautions taken to use animals which have no complicating pulmonary diseases. The role of posture should be investigated by exposing animals to irritant gases while suspending them in vertical positions during and after exposure. More sophisticated studies of the production and composition of silo gas should be conducted. The role of antigen-antibody interactions in the development of BFO and the chronic effects of exposure demand investigation. Perhaps the most important, as well as overlooked, problem for investigation is the evaluation of the effects of repeated subclinical exposures to silo gas.

SUMMARY

Research was conducted to determine the composition of silo gas, the effects of silo gas in pigs, and the similarity between the lesions of silo gas toxicosis in pigs, nitrogen oxides toxicosis in rats, and silo filler's disease in man.

The analysis of gas was made with the mass spectrometer. The concentrations in 2 silos were: (a) carbon dioxide, 0.173 to 9.054%, (b) nitric oxide, 0 to 140 parts per million, and (c) nitrogen dioxide, 8 to 360 parts per million.

Pigs were exposed for 3, 4, 5, and 10 minutes to silo gas. All pigs exposed for 10 minutes died within 21 to 72 minutes. All other pigs survived exposure, with the exception of 1 pig exposed for 4 minutes, which died in 10 hours and 27 minutes. Survivors were killed and examined at 15, 29, 30, 60, and 61 days.

In pigs dying from exposure to silo gas, the earliest lesions noted were alveolar edema and hyperemia. The edema was eosinophilic and formed large quantities of fibrin in pigs that survived the longest. In addition, there was hemorrhage, bronchiolar and bronchial epithelial desquamation, and perivascular, interlobular, and subpleural edema. These lesions were similar to those produced in rats by exposure to high concentrations of nitrogen oxides. Pigs surviving the acute effects of silo gas toxicosis did not develop bronchiolitis fibrosa obliterans, which has been described for silo filler's disease in man.

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