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BOVINE PULMONARY ADENOMATOSIS  
INDUCED BY NITROGEN DIOXIDE

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by

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## INTRODUCTION

Bovine pulmonary adenomatosis is a disease of cattle characterized clinically by progressive dyspnea, usually terminating in death and pathologically by pulmonary epithelial hyperplasia, emphysema and edema.

The cause of this disease is not known. Bacterial and fungal toxins, viral infections and allergens have been discussed as possible causes (21).

Bovine pulmonary adenomatosis may be the result of inhalation of nitrogen dioxide produced in the rumen during an abnormal fermentation process. Several factors have led to this conclusion. They are:

1. The experimental production of the disease in a cow by the inhalation of oxides of nitrogen, (nitrogen dioxide)(23).
2. The occurrence of a disease with similar lesions in man, called silo filler's disease, caused by the inhalation of nitrogen dioxide gas produced in recently filled silos (9, 10).
3. An increased occurrence of the disease in cattle following a major change in the ration, known to alter fermentation processes in the rumen (14, 15, 23).
4. A higher incidence of the condition in man and cattle during droughts (15, 22) when the nitrate content of immature plants is increased (9, 10).
5. The heavy use of high nitrogen content fertilizers on crop lands and pastures, known to increase plant nitrates (10).
6. The similarity, in certain respects, of fermentation processes in the rumen and in silos.

7. The findings of Dougherty et al. (6, 7) that pharyngeal and laryngeal activities during eructation in ruminants facilitate the entrance of eructated gases directly into the trachea and subsequently into the lungs.

With this eructation-inhalation mechanism, it seems possible that nitrogen dioxide, or other toxic gases, produced in the rumen during fermentation of plants high in nitrates could cause serious lung damage.

This project was designed to study and describe the clinical and pathologic alterations produced in the bovine by nitrogen dioxide gas administered by various routes, including intraruminal, intratracheal, and forced inhalation. Intraruminal exposure was accomplished through a rumen fistula so there would be no interference with the eructation process as occurs during intubation. This interference may have been involved in the attempt by Seaton (23) to produce the disease by intraruminal exposure. Different sources of nitrogen dioxide were used to determine if impurities were involved in previously reported work with this gas. Nitric acid vapor was also used to determine if reduction of this acid to nitrogen dioxide was necessary for the development of lesions of pulmonary adenomatosis. The methods, results, and conclusions of this study follow.

## REVIEW OF LITERATURE

Bovine pulmonary adenomatosis was first described in 1953 in Texas by Monlux et al. (14). They reported the condition in association with moldy feeds, including corn stalks, sweet potatoes, milo and higari. It occurred in several herds of cattle during the fall of 1952 and had been observed by one of the authors in 1932 in several herds feeding on sweet potatoes affected by black rot. The disease was usually fatal after clinical signs were observed, but new cases did not occur after discontinuance of the moldy feed.

In 1955 the disease was reported in Iowa cattle, again by Monlux et al. (15). This report, together with reports by Seaton (22, 23), described the clinical and pathologic alterations, incidence and field conditions under which the disease occurs. Except for association of the condition in Texas with the consumption of moldy feeds, descriptions of the disease by these authors are similar and include the following major points.

Bovine pulmonary adenomatosis is a disease of cattle without age, sex or breed predisposition. It is most commonly seen following a sudden change in the ration such as occurs when animals are put in feed lots on full feed or on lush green pasture, often alfalfa. The condition has been observed most frequently in yearling and two-year-old animals. One or several members of a herd may be affected. Most cases are seen during the summer and fall months and the incidence is greatly increased following a spring and summer of decreased rainfall.

Clinically, the disease is characterized by sudden occurrence; usually terminating in death. Severely affected animals often die within twenty-four hours. Those less severely affected that die, usually do not live beyond five days, death occurring most frequently the third day after onset of symptoms.

The first and most characteristic sign of the disease is severe dyspnea, seen as unusually deep and rapid respirations. As the disease progresses the dyspnea becomes more prominent until the animal stands with its mouth open and neck extended in an attempt to obtain sufficient oxygen. Abdominal breathing becomes progressively more evident and a characteristic loud expiratory grunt frequently develops. Auscultation of the thorax reveals areas of consolidation of the lungs where no sounds are present, and other areas where rales can be heard. Tachycardia is prominent and the animal may salivate excessively. Body temperature may remain normal or may be slightly elevated, especially during "warm weather." There is rapid dehydration and moderate loss of weight. Subcutaneous emphysema of the dorsal cervical and thoracic regions is occasionally seen. A consistent finding, which is helpful in differential diagnosis, is the failure to respond to any known therapy.

The important gross post mortem changes are confined primarily to the lungs. They are greatly enlarged, often with the imprint of the ribs on their surface, and they do not collapse upon opening the thorax. Consolidation of the lungs is either diffuse or more often alternating with multiple areas of alveolar and interstitial emphysema. The areas of consolidation have a reddish-pink slightly cyanotic appearance and are

very firm and heavy. The trachea and bronchi often contain white foam indicating terminal pulmonary edema. Interstitial emphysema of the lungs is common with "large bullae" frequently present in the interlobular and subpleural tissue. The mediastinum as well as the mediastinal and bronchial lymph nodes are frequently distended with "pockets of gas." Gas may also be found in the subcutaneous tissue of the cervical and dorsal thoracic regions and in the perirenal tissue. In prolonged cases, a complicating bronchopneumonia is sometimes present in the antero-ventral lobules. The right cardiac ventricle is usually dilated and there is general passive hyperemia.

Microscopic examination of the lungs reveals characteristic changes, the most prevalent being hypertrophy and hyperplasia of the "septal cells." These cells are seen lining the alveoli, as well as free in the lumens. There are hypertrophy and hyperplasia of the bronchial epithelium, with many of the bronchi filled with desquamated cells. The alveoli and bronchi may also contain "edema fluid" and small amounts of fibrin. Alveolar and interstitial emphysema are common and prominent. Acute passive hyperemia and mild fatty degeneration are usually present in other "parenchymatous organs."

Since treatment of this condition with many therapeutic agents has not been successful, it is imperative that other measures be practiced. The most favorable results have been obtained by changing the diet of the animal along with prevention of undue excitement.

Seaton (23) states that pulmonary adenomatosis has been diagnosed in cattle originating in Minnesota and Kentucky as well as Texas and Iowa

and probably occurs in other midwestern states. In 1960 Vickers et al. (26) reported the condition in several herds of South Carolina cattle feeding on a variety of material including lush fescue, rye and oat pastures, poor permanent pasture, as well as moldy sweet potatoes, soybean hay and peanut hay.

A number of diseases resembling bovine pulmonary adenomatosis have been observed in cattle and other species. A summary of several of these has been assembled by Seaton (22, 23). Acute bovine pulmonary emphysema, which occurs in the Rocky Mountain Region, appears similar if not identical to adenomatosis. However, the cause of neither disease is known and until determined, their semblance will remain obscure. Other names such as panthers, bovine asthma, atypical interstitial pneumonia and idiopathic pulmonary emphysema have been used to describe pulmonary conditions in cattle which are clinically and pathologically similar to the adenomatosis-emphysema complex. An extensive literature review of acute bovine pulmonary emphysema and related conditions in cattle has been compiled by Maki (12).

Nitrogen dioxide poisoning in cattle, mice and guinea pigs has been studied by Seaton (23). The gas was produced by the reduction of nitric acid by copper electrical wire. Typical lesions of pulmonary adenomatosis developed in a cow following two exposures by inhalation but did not develop when the toxic gas was given repeatedly through a stomach tube into the rumen. These same characteristic lesions were found in mice and guinea pigs following only one exposure to nitrogen dioxide.

Nitrogen dioxide poisoning in man has been reported on several occasions. Spencer (24) stated that the first full description of the

effects on the human lung was given by Nicholas in 1930 following a fire in which large quantities of nitrocellulose X-ray film underwent slow combustion. Grayson (9), and Lowry and Schuman (10) in 1956 and Rafii and Godwin (20) in 1961 reviewed the literature and gave detailed descriptions of nitrogen dioxide poisoning in man. Their studies were confined primarily to silo filler's disease caused by inhalation of nitrogen dioxide produced by the fermentation of ensilage with a high nitrate content.

## METHOD OF PROCEDURE

## Materials

Animals

Twelve Holstein-Friesian heifers from the National Animal Disease Laboratory herd were used for this study. They ranged in age from ten to nineteen months. There was neither history nor clinical signs of respiratory disease.

Gas supply

The nitrogen dioxide was from two sources, including commercial gas<sup>1</sup> and that produced by reduction of concentrated nitric acid with metallic copper (sheet copper and copper electrical wire). Nitric acid vapor was produced by heating concentrated nitric acid.

Rumen cannulas

Rumen cannulas were made by the Physiopathological Investigations at the National Animal Disease Laboratory as described by Dougherty (5). The barrel of each cannula was made of rubber laboratory tubing, 2.5 cm. in diameter by 12 cm. in length. One end was vulcanized to a 0.64 by 7.5 cm. rubber washer<sup>2</sup> ( $\frac{1}{4}$  in. sheet rubber, packing grade #1172). A 12 cm. rubber washer<sup>2</sup> was made to slip over the portion of tube protruding from the rumen. At the time of gas administration, cotton gauze

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<sup>1</sup>The Matheson Company, Inc., Joliet, Illinois.

<sup>2</sup>The B. F. Goodrich Company, Akron, Ohio.

and Elastic Putty<sup>1</sup> were put under the outer rubber washer and a stiff Plexiglas<sup>2</sup> ( $\frac{1}{4}$  in. G grade) washer, 0.64 by 12 cm., was placed over it to prevent leakage of gas.

#### Surgical preparation of animals

Four animals were surgically equipped with rumen cannulas for introduction of gas. Tracheotomies were performed on four others and a tube inserted in each for introduction of gas directly into the trachea. The remaining four needed no preparation.

Surgical preparation consisted of clipping the hair, scrubbing the area with Septisol<sup>3</sup> (liquid soap containing hexachlorophene), and application of 1:750 concentration of 17 per cent Zephiran Chloride<sup>4</sup> (benzylkonium chloride). The line of incision was infiltrated with four per cent procaine.

The rumen cannula operation (5) was conducted in two steps. Step one consisted of making a vertical incision, approximately 6 cm. in length, through the abdominal wall in the left paralumbar fossa midway between the os coxae and last rib. The incision was made as close as possible to the transverse processes of the lumbar vertebrae. Ends of the incision were rounded by removal of a small section of skin. The rumen was then pulled through the incision for 3-4 cm. and sutured to

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<sup>1</sup>Montgomery Ward, Chicago, Illinois.

<sup>2</sup>Cadillac Plastic and Chemical Company, Chicago, Illinois.

<sup>3</sup>Vestal, Inc., St. Louis, Missouri.

<sup>4</sup>Winthrop Laboratories, New York, New York.

the skin. Staple stitches were used, trying not to penetrate into the lumen of the rumen. The exposed rumen was covered with unbleached muslin coated with petrolatum. After sufficient time (2 weeks) for adequate adhesion to occur between the rumen and abdominal wall, step two was performed. The exposed section of the rumen was removed and a cannula with attached inner rubber washer was inserted. Sulfanilamide was applied to the exposed area around the cannula. The outer cannula washer was put in place and secured with adhesive tape. After complete healing of the ruminal incision, the exterior disc of the cannula was removed and the area was scrubbed with soap and water. Scrubbing was performed as indicated by the degree of contamination of the area around the cannula.

The tracheal tube operation consisted of making an incision, approximately 2 cm. in length, over the trachea in the mid-ventral cervical region. The muscles were separated by blunt dissection and a stab incision was made through the wall of the trachea. A Nalgon<sup>1</sup> (polyvinyl chloride) tube, 6 mm. in diameter, was inserted into the lumen of the trachea for a distance of approximately 8 cm. The tube was anchored by a ligature tightly fastened around it and the ends used to close the skin incision. A small opening was left at the ventral edge of the incision for drainage. The protruding end of the tube was fastened to the cervical skin by adhesive tape. This tube was placed in the trachea the day prior to exposure.

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<sup>1</sup>The Nalge Company, Inc., Rochester, New York.

## Exposure to Nitrogen Dioxide

Intraruminal exposure

Commercial nitrogen dioxide was administered to four animals via the rumen. A Nalgon tube was attached to a needle valve on the gas cylinder and the gas was forced through the paralumbar cannula into the rumen.

To be assured of a steady flow rate, the gas was heated to 37° C. by placing the container in a water bath at that temperature. This was necessary, since the boiling point of nitrogen dioxide (21.2° C.) (27) is only slightly below room temperature.

An attempt was made to administer a continuous flow of gas to two animals. It was estimated by use of a flow meter, that each received a total of 15000 ml. during the first day. Because of unsatisfactory control over dosage, this method of administration was discontinued in favor of daily doses of gas. No attempt was made to measure the amount given and both animals died the third day, shortly after receiving large doses.

The other two animals of this group were given daily doses of gas over a period of 55 days. Dosages were controlled by regulating the time (20-30 sec.) of administration at a constant gas flow rate. Starting with small doses, the amount of gas given was gradually increased until the methemoglobin levels of the blood, one-half hour after exposure, approached but did not exceed one-third the total hemoglobin levels. After the dosage was established, a constant amount of gas was given each animal. At the end of the 55-day exposure period, they were electrocuted.

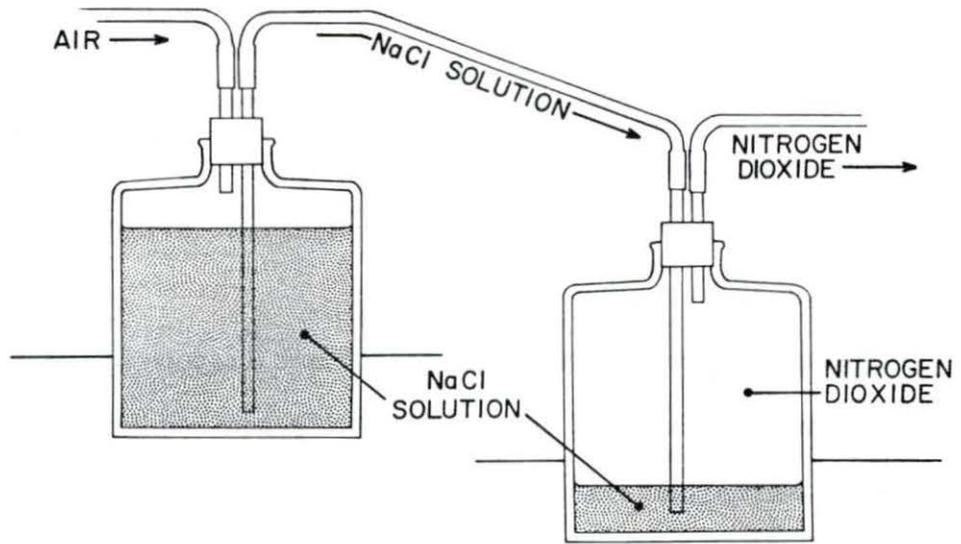
### Intratracheal exposure

Four animals were given daily doses of nitrogen dioxide gas by the tracheal tube route. Two of these were given commercial gas, one the gas evolved from the reduction of nitric acid by sheet copper, and one the gas evolved from the reduction of nitric acid by copper electrical wire.

The gas was forced from a large bottle into the tracheas over a five-minute period. The apparatus in which the gas had previously been collected consisted of an airtight two-bottle siphoning system containing a saturated solution of sodium chloride (Fig. 1). By lowering or elevating one bottle, gas could be collected in or forced from the other bottle. When collecting gas, an airtight seal was made between the gas source and collecting bottle to prevent the entrance of air.

These four animals were given gas daily until death occurred or appeared certain. They lived for periods of from 11 to 25 days after initial exposure. Methemoglobin levels of the blood, following initial small doses of gas, were again used to adjust the dosage rates. The siphoning bottles were used to accurately measure the amount of gas given. Each animal was given an average daily dose of 1600 ml. with the lowest initial dose being 200 ml. and the highest terminal dose being 6000 ml. The terminal individual dose varied from 2000 to 6000 ml.

Fig. 1. Siphoning apparatus for measuring gas volume. By lowering or elevating either bottle, gas can be collected in or forced from the other



An equilibrium, dependent on temperature, exists between nitrogen dioxide and its dimer, dinitrogen tetroxide (3); so to assure correct measurement at body temperature, the commercial gas was heated to 37° C. With gas generated by reduction of nitric acid by metallic copper, no additional heat was needed because of the exothermic nature of the reaction.

#### Forced inhalation exposure

Two animals were forced to inhale the toxic gas directly. One was given the gas evolved from reduction of nitric acid by sheet copper and the other the gas evolved from reduction of nitric acid by copper electrical wire.

For administration of gas, one end of a burlap bag, open at both ends, was placed over the animal's nose and tightly secured to the chin and nosepiece of the halter (21). The flask containing the copper and nitric acid was held under the open end of the bag, thus creating a concentration of gas in the immediate vicinity of the external nares.

Measurement of gas given was not possible by this method of administration. Gas was given for periods of one to two minutes every third to fourth day until death occurred. One animal was exposed only once while the other was forced to inhale the gas four times over a period of 15 days. The one given gas only once died two days later, while the other died after exposure on the fifteenth day.

#### Exposure to Nitric Acid Vapor

Two animals were forced to inhale nitric acid vapor. This was administered by the same procedure as used for nitrogen dioxide, by

boiling concentrated nitric acid under the open end of a burlap bag placed around the animal's nose. Each was exposed to the vapor for approximately four minutes and electrocuted three days later.

### Clinical Procedure

#### Observations

Observations of the general health status of the animals were made several times daily. Individual changes in respiration, feed consumption, discharges, excreta and general appearance, as well as any other changes were recorded. The temperatures and respiratory rates were observed and recorded shortly before and one-half hour after the administration of gas.

#### Hematology

The animals were bled twice daily for determination of hemoglobin and methemoglobin levels and for total and differential leukocyte counts. Potassium oxalate was used as the anticoagulant.

Total leukocyte counts were made daily. Blood smears were prepared daily and fixed in methyl alcohol for later differential counting. They were stained for 30 minutes with Giemsa's blood stain diluted with 20 parts distilled water.

Hemoglobin determinations were made at weekly intervals, starting the day prior to the administration of gas. These determinations were used for comparison with and for calculation of methemoglobin levels of the blood. Hemoglobin determinations were made by the cyanmethemoglobin method (4).

Methemoglobin levels were determined twice daily, just prior to and one-half hour following the administration of gas. They were determined by the spectrophotometric procedure of Evelyn and Malloy (8), as follows:

1. Mix 0.1 ml. of oxalated blood with 10 ml. of M/60 phosphate buffer at pH 6.6.
2. Allow to stand for 5 minutes.
3. Determine optical density at 635  $\mu$ ., using distilled water as the blank, =  $D_1$ .
4. Add 1 drop of neutralized sodium cyanide to entire 10 ml. sample.
5. Allow to stand for 2 minutes.
6. Make second reading at same wave length, =  $D_2$ .
7.  $(D_1 - D_2) \times F_m =$  grams of methemoglobin per 100 ml. of blood.

$F_m$  is a proportionality constant determined by the following procedure:

1. Determine the hemoglobin content of normal blood.
2. Add 0.1 ml. of blood to a mixture of 9.9 ml. of M/60 phosphate buffer at pH 6.6 and 0.1 ml. of 5 per cent potassium ferricyanide.
3. Mix and allow to stand for 2 minutes.
4. Determine optical density at 635  $\mu$ ., using a mixture of 10 ml. of the phosphate buffer and 0.1 ml. of the ferricyanide as the blank, =  $D_1$ .
5. Add 1 drop of neutralized sodium cyanide solution to entire sample and to the blank.
6. Mix and allow to stand for 2 minutes.

7. Determine the density against the blank at the same wave length, =  $D_2$ .
8.  $F_m = \frac{\text{hemoglobin content in grams per 100 ml.}}{D_1 - D_2}$

The neutralized sodium cyanide was prepared as follows:

1. Prepare 10 per cent sodium cyanide.
2. Prepare 12 per cent acetic acid.
3. Add the 12 per cent acetic acid to the 10 per cent sodium cyanide in the ratio of 1 to 1 to make the final product.
4. The solution is made fresh each time it is to be used.

#### Necropsy Procedure

Immediately following spontaneous death or electrocution, thorough necropsies were performed. All gross lesions were described and the following tissues were taken from each animal for microscopic examination.

#### Cardiovascular system

Ventricular septum and papillary muscle of the heart.

#### Digestive system

Tongue	Ventral rumen wall	Cecum
Submaxillary salivary gland	Omasum	Colon
Parotid salivary gland	Abomasum	Rectum
Esophagus	Duodenum	Liver
Reticulum	Jejunum	Gall bladder
Dorsal rumen wall	Ileum	Pancreas

Endocrine system

Adrenal gland

Pituitary gland

Thyroid gland

Lymphatic system

Bronchial lymph node

Prefemoral lymph node

Cervical lymph node

Prescapular lymph node

Hepatic lymph node

Retropharyngeal lymph node

Internal iliac lymph node

Submaxillary lymph node

Mediastinal lymph node

Supramammary lymph node

Mesenteric lymph node

Spleen

Parotid lymph node

Thymus

Muscular system

Skeletal muscle from pectoral region

Nervous system

Anterior cerebral cortex

Midbrain

Posterior cerebral cortex

Anterior medulla oblongata

Cerebellar cortex

Posterior medulla oblongata

Corpus striatum

Cervical spinal cord

Thalamus

Thoracic spinal cord

Hippocampus

Lumbar spinal cord

Respiratory system

Right and left dorsal turbinates

Larynx

Nasopharynx

Mid-trachea

Tracheal bifurcation

Dorsal and ventral regions of right and left apical lobes of the lungs

Dorsal and ventral regions of right and left cardiac lobes of the lungs

Intermediate lobe of the lungs

Anterior and posterior regions of right and left diaphragmatic lobes of the lungs

Mid-lateral and medial regions of right and left diaphragmatic lobes of the lungs

#### Urogenital system

Kidney

Ovary

Urinary bladder

Uterus

Tissues, 6 to 10 mm. thick, taken for microscopic examination were submerged in formalin for not less than 48 hours. Ten per cent formalin was used for all except nervous tissue, in which case 25 per cent was used. The tissues were trimmed, dehydrated in graduated concentrations of ethyl alcohol, cleared in Clearing Agent<sup>1</sup> and infiltrated in Paraplast<sup>2</sup> in the routine manner in an Autotechnicon.<sup>1</sup> Sections were embedded in Paraplast, sectioned at eight microns and mounted on glass slides for staining.

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<sup>1</sup>The Technicon Company, Chauncey, New York.

<sup>2</sup>Aloe Scientific, St. Louis, Missouri.

All sections were stained in a routine manner with Mayer's hematoxylin and eosin Y. Sections of the lungs were also stained by Weigert's technic for demonstrating fibrin. Liver sections were stained with oil red O in propylene glycol for fat and by the periodic acid-Schiff method for glycogen. Sections of skeletal and cardiac muscle were stained with Gomori's one-step trichrome stain to demonstrate necrosis. Staining procedures are described in the Armed Forces Institute of Pathology, Manual of Histologic and Special Staining Technics (1).

## FINDINGS

On the basis of clinical observations and tissue alterations, the experimental animals can be divided into three groups, consistent with the route of gas administration and type of gas given. These groups include; first, those given nitrogen dioxide intraruminally, second, those given nitrogen dioxide directly into the lungs either by forced inhalation or by tracheal tube, and third, those given nitric acid vapor. No consistent difference was found between those given commercial nitrogen dioxide and those given nitrogen dioxide evolved by mixtures of nitric acid and copper electrical wire or sheet copper.

## Clinical Findings

Clinical effects of exposure to nitrogen dioxide via the rumen

The four animals given nitrogen dioxide intraruminally had much less discomfort than those given the gas directly into the lungs, either via the tracheal tube or by forced inhalation. Intraruminal administration caused the animals to kick at the abdomen and stamp on the floor as is observed in acute abdominal pain. They became only slightly excited and showed little resistance to restraint. Upon eructation the brownish nitrogen dioxide gas coming from the mouth and nostrils caused considerable salivation and lacrimation; accompanied by head shaking, yawning movements, and occasionally coughing. No respiratory change, other than coughing, occurred during gas administration.

Respiratory rates varied according to the route of gas administration. Each animal exposed intraruminally developed a progressive decrease

in respiratory rate without an increase in tidal flow. The rates in these animals one-half hour after gas administration was only slightly increased over the pre-exposure rates (Fig. 2). This is in sharp contrast to those given the gas by tracheal tube or forced inhalation (Fig. 3).

The temperatures of all animals of this group, except one, remained fairly constant and within the normal range throughout the exposure period. One developed slight fever, probably the result of complications other than exposure to the toxic gas.

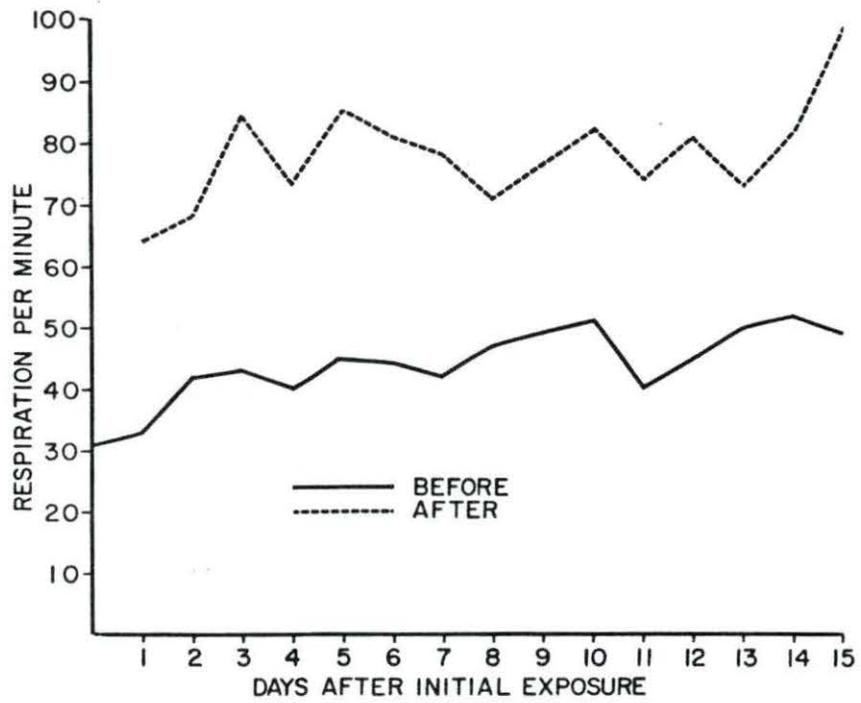
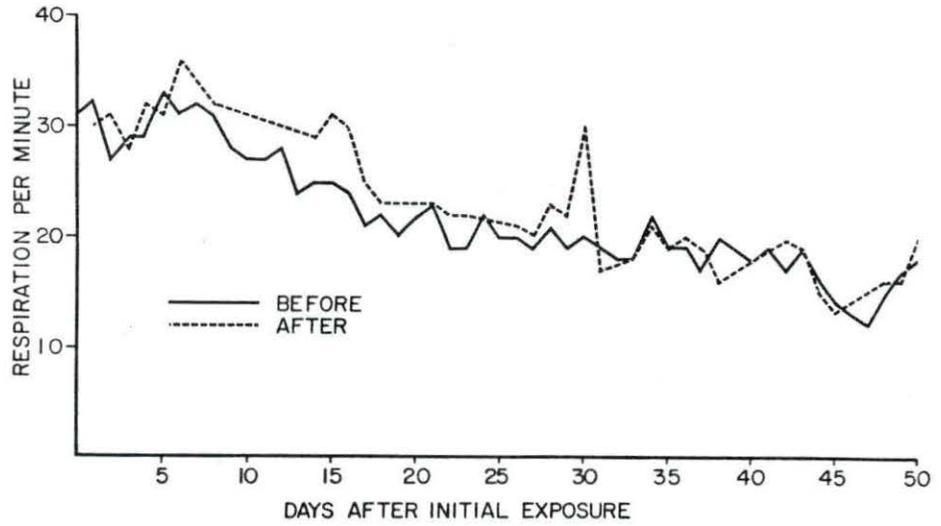
Feed and water consumption decreased rapidly following initial exposures. The appetites remained poor and erratic until the animals died or were electrocuted. Rumen motilities were greatly decreased and at times complete stasis occurred. Evacuations were scanty but normal in consistency. Slight mucous nasal discharge developed which varied considerably from day to day. The two animals given gas over a prolonged period of time rapidly became dehydrated and emaciated.

Those given large intraruminal doses on the third day suddenly became recumbent, very cyanotic and comatose; developed rapid, deep, respirations and running motions which progressed into tetany. One died in ten minutes and the other one hour after exposure; the latter having been in coma the day prior to death as a result of an excessive dose of nitrogen dioxide.

The methemoglobin levels of the blood one-half to one hour after exposure to nitrogen dioxide were used as an effective indication of dosage, regardless of route of administration. This was found to be

Fig. 2. Average daily respiratory rates before and after intraruminal exposure to nitrogen dioxide

Fig. 3. Average daily respiratory rates before and after inhalation of nitrogen dioxide



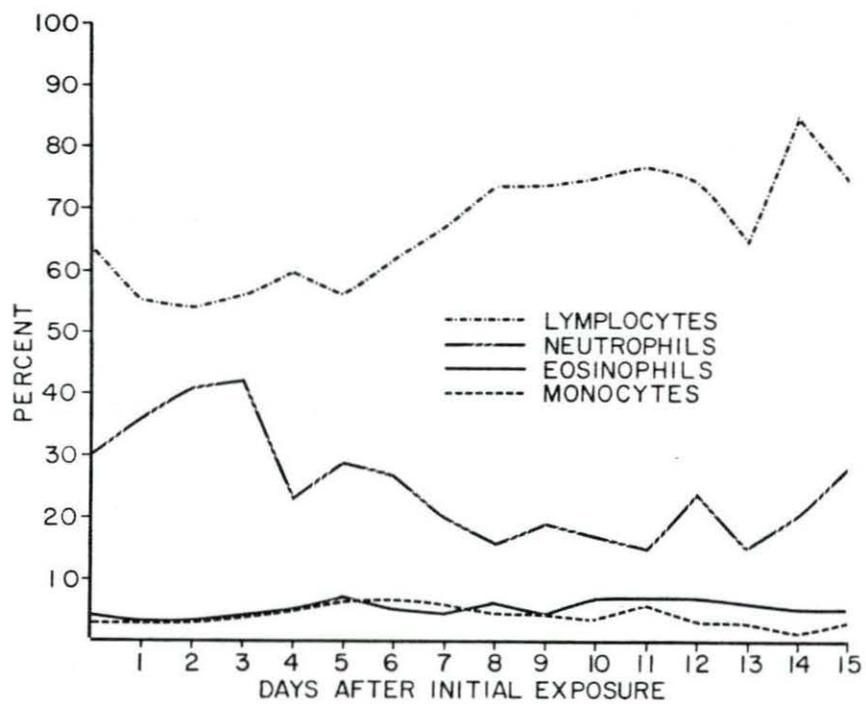
the time of optimal methemoglobinemia and corresponded well with the doses of gas employed. Depending upon the initial concentration of methemoglobin, the blood level would return to normal within from 12 to 24 hours after exposure. Methemoglobin levels in general did not exceed 4 mg/100 ml. of blood which represented about one-third the total hemoglobin. Methemoglobin levels of the two that died suddenly after large intraruminal doses of nitrogen dioxide were approximately equal to the normal hemoglobin concentrations. When the methemoglobin concentration approximated one-half the normal hemoglobin level, the animal became very weak and incoordinated. Coma resulted in one when the methemoglobin level reached approximately three-fourths the total hemoglobin.

Total leukocyte counts of all animals, regardless of gas given or route of administration, remained constant throughout the experimental period. On the other hand, differential leukocyte counts (Fig. 4) of all animals exposed to nitrogen dioxide showed a trend toward an increase in lymphocytes and a slight decrease in neutrophils. No changes were noted in the numbers of eosinophils or monocytes or in the proportion of segmented to non-segmented neutrophils.

#### Clinical effects of exposure to nitrogen dioxide via tracheal tube or by forced inhalation

In contrast to the reaction of the animals during administration of nitrogen dioxide intraruminally, those given the gas directly into the lungs became extremely excited and struggled violently. This was more exaggerated in those receiving the gas by forced inhalation than

Fig. 4. Average daily differential leucocyte count of all animals exposed to nitrogen dioxide



by the tracheal tube route. At the beginning of the administration period, there was apnea for short intervals; after which, following inspiration, considerable coughing would frequently ensue. Then toward the end of the exposure period, respirations became deep and rapid. Lacrimation and salivation were more copious than with intraruminal exposure.

Again, in contrast to the animals exposed intraruminally, both the pre-exposure and post-exposure respiratory rates of those exposed directly into the lungs showed a progressive increase throughout the experimental period. Post-exposure rates of these animals was approximately double pre-exposure rates (Fig. 3). Coughing, other than during gas administration, was not a consistent or frequent finding. Auscultation of the thorax revealed rales and areas of consolidation several days prior to death.

As death approached, the animals would develop an apprehensive appearance, probably as a result of inability to obtain sufficient oxygen. They would stand with their mouths open and tongues protruding in an effort to get enough air. Respirations would become very deep with a characteristic double expiratory effort accompanied by a loud grunt or groan.

Body temperatures remained normal or were only slightly elevated. Secondary pulmonary infections were no doubt the cause of the slight fever.

As with the intraruminally exposed group, the feed and water consumption fell rapidly after initial exposure to gas. This was erratic and remained low until complete inappetance developed a few

days prior to death. Rumen motilities were decreased but complete atony did not occur until just prior to death.

All of this group rapidly became dehydrated and emaciated. Mucous to muco-purulent nasal discharges developed and became progressively more abundant until death occurred.

The post-exposure methemoglobin concentrations as well as the total and differential leukocyte counts (Fig. 4), corresponded closely to those of the intraruminally exposed group.

#### Clinical effects of exposure to nitric acid vapor

The two animals forced to inhale nitric acid vapor reacted similarly to those forced to inhale nitrogen dioxide gas. Both became very excited and struggled violently during administration. Lacrimation and salivation were excessive but not as abundant as with nitrogen dioxide.

The respiratory rates were also similar to those during forced inhalation of nitrogen dioxide, the post-exposure rates being approximately double the pre-exposure rates. No respiratory signs were observed other than the changes during and immediately after administration of the vapor.

The temperature, appetite, and general appearance of each remained normal until they were electrocuted three days after exposure.

No increase in blood methemoglobin levels were found following exposure to the vapor.

## Necropsy Findings

Lesions of exposure to nitrogen dioxide via the rumen

Cardiovascular system      General passive hyperemia was grossly prominent in the two acutely poisoned animals. The blood was dark brown in color. Small petechiae were present in the epicardium of each animal and the right ventricle of one was dilated.

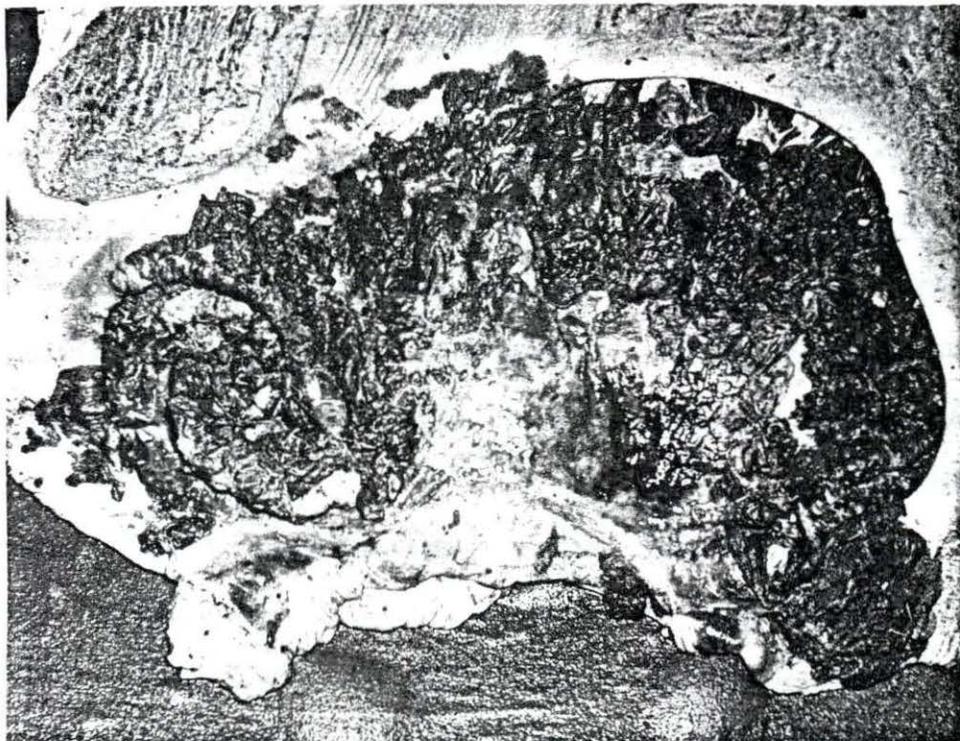
Microscopically there were multiple foci of coagulative necrosis and diffuse swelling of the myocardium. A few hemorrhages were present in the necrotic areas.

The heart of one chronically poisoned animal was greatly dilated but no microscopic changes, other than mild general passive hyperemia, were found in either.

Digestive system      The principle gross lesion of the digestive systems was produced by the action of the gas directly on the rumens. The rumen around the fistula of each acutely affected animal was very thick and edematous, having a brownish-yellow color similar to that of the gas. Only small areas of the mucosa had sloughed. The dependent portions of the intestines were very hyperemic. Gross lesions of those chronically affected were similar but much more prominent. Large areas of the ruminal mucosa, 25-30 cm. in diameter, were necrotic, much of which had sloughed leaving a very hyperemic, inflamed and edematous submucosa (Fig. 5). One animal also had a moderately enlarged (atonic) omasum.

Microscopically there was extensive coagulative necrosis of the rumens in the vicinity of the cannulas. Leucocytes were abundant under the necrotic tissue. There was extensive sloughing of the ruminal

Fig. 5. Necrosis of rumen following prolonged intraruminal exposure to nitrogen dioxide



epithelium adjacent to the cannulas in the chronically affected animals and severe edema of the same region in those acutely affected. Mild cloudy swelling of the hepatic parenchyma, especially of the centrolobular regions, was observed in the chronically affected animals. Passive hyperemia was observed in all digestive tissues examined.

Endocrine system No change other than passive hyperemia was seen in the pituitary, adrenal, thyroid, or pancreatic glands.

Lymphatic system Little change was found in the lymphoid tissue grossly. Numerous petechiae were present in the thymus of one acutely affected animal and there appeared to be hyperplasia of the lymphoid tissue of the pharyngeal and retropharyngeal regions of those chronically poisoned.

Microscopically the major change was focal accumulations of blood in the lymph node sinuses. These foci were widespread in the lymph nodes of the acutely affected animals but were found in only a few nodes of those chronically affected. One acutely affected animal also had widespread reticuloendothelial cell hyperplasia of the lymph nodes, which apparently was not associated with the nitrogen dioxide gas.

Muscular system Skeletal muscles of the acutely poisoned animals were dark brownish red as a result of the action of nitrogen dioxide on myoglobin and hemoglobin. Musculature of those chronically affected appeared unaltered grossly.

Microscopically the skeletal muscles were diffusely swollen and contained areas of early degenerative changes.

Nervous system      No gross alterations were found in the brains or spinal cords.

A few small hemorrhagic foci and mild passive hyperemia were found on histologic examination of the brains.

Respiratory system      Little gross change, other than mild general hyperemia and a few petechiae in the nasopharyngeal regions of the acutely poisoned animals, was seen in the upper respiratory tracts.

Microscopically, there was mild leucocytic infiltration of the mucosa and submucosa of the tracheas, as well as passive hyperemia. One acutely poisoned animal also had a few foci of hemorrhage in the submucosa and a thin layer of fibrin lining the trachea.

Grossly the lungs of the acutely poisoned animals were mottled with a few focal areas of consolidation mixed with areas of emphysema; whereas, the lungs of those chronically poisoned appeared normal.

Microscopically the pulmonary lesions were similar in both the acutely and chronically affected animals, and were consistent in all lobes of the lungs. Each had hypertrophy and hyperplasia of the bronchial and bronchiolar epithelium, the epithelium being tall columnar in the former and stratified columnar to squamous in the latter (Fig. 6). In a few bronchioles the epithelium completely filled the lumens. The musculature of the bronchi and bronchioles was slightly hyperplastic. The peribronchial lymphoid tissue and mucous glands were moderately hyperplastic. There was diffuse active hyperemia with more blood present in some lobules than others. Several areas of alveolar emphysema and others of atelectasis, with a definite lobular pattern, were present (Figs. 7, 8).

Fig. 6. Hyperplasia, hypertrophy and desquamation of bronchial epithelium with hemorrhage and lymphocytic infiltration following three daily intraruminal exposures to nitrogen dioxide.  
Mayer's hematoxylin and eosin Y. x 100



The atelectatic lobules were more common in the chronically poisoned animals. Interstitial emphysema with moderate distension of the lymphatics was also a common finding (Fig. 7). In addition, the lungs of the acutely poisoned animals contained numerous small deposits of fibrin. Much of the fibrin was seen lining the alveoli and occasionally the bronchioles and bronchi. Pulmonary edema, again with a somewhat lobular pattern, was also a prominent feature of the lungs of these two animals. An occasional focus of hemorrhage was present and a macrophage was rarely found in the lumen of an alveolus. No fibrin, edema, hemorrhage or macrophages were found in the chronically affected animals.

Urogenital system      Grossly the kidneys were dark red because of a large amount of blood.

Microscopic examination of the kidneys revealed multiple focal areas of coagulative necrosis of the proximal convoluted tubules of the two chronically poisoned animals and one of those acutely poisoned (Fig. 9, 10). There was only slight degenerative change in the other acutely affected animal. The blood vessels in and surrounding the necrotic areas were greatly distended with blood.

Lesions following exposure to nitrogen dioxide  
by tracheal tube or by forced inhalation

Cardiovascular system      Both gross and microscopic changes were inconsistent. Extensive pericardial emphysema (Fig. 11) of two animals and multiple ecchymotic hemorrhages in the pericardium of another were seen grossly. Excessive straw-colored fluid was found in the peritoneal and pleural cavities and the pericardial sacs of two animals.

Fig. 7. Alveolar and interstitial emphysema following three daily intraruminal exposures to nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 100

Fig. 8. Atelectasis surrounding a bronchiole with hyperplastic epithelium following 55 daily intraruminal exposures to nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 125

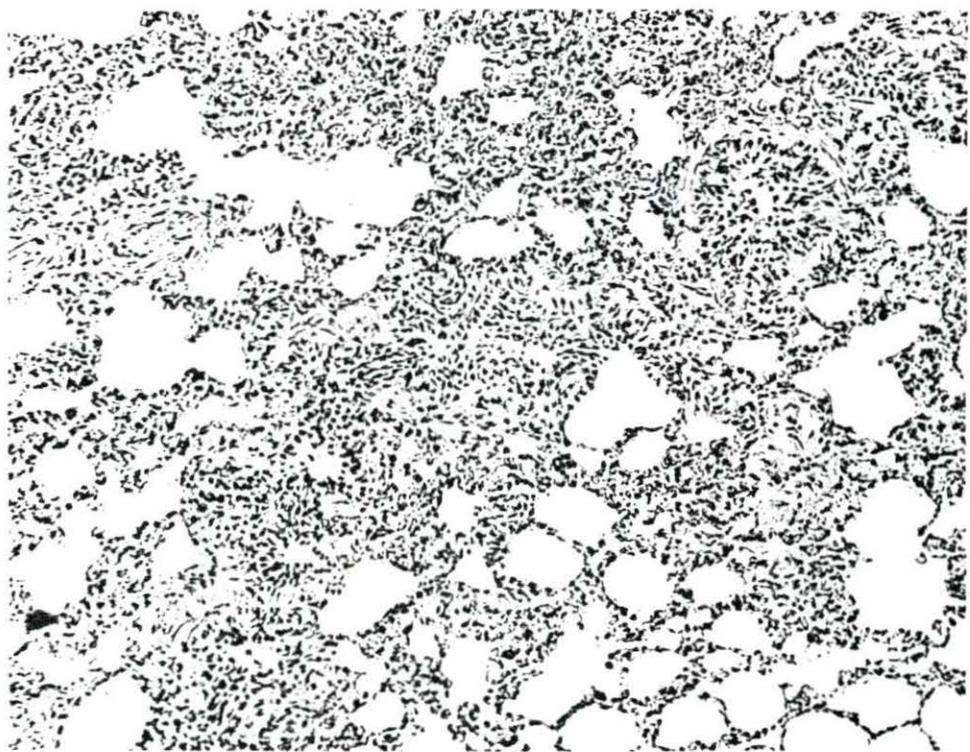
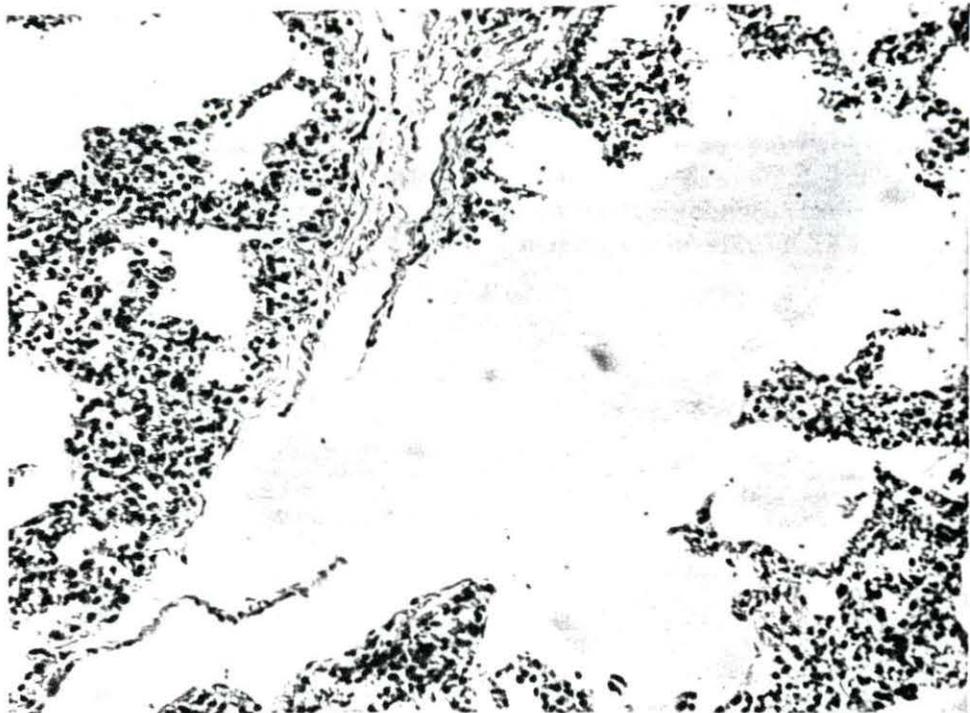


Fig. 9. Diffuse coagulative necrosis of proximal convoluted tubules with severe passive hyperemia following three daily intraruminal exposures to nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 20

Fig. 10. Higher magnification of Fig. 9  
x 125

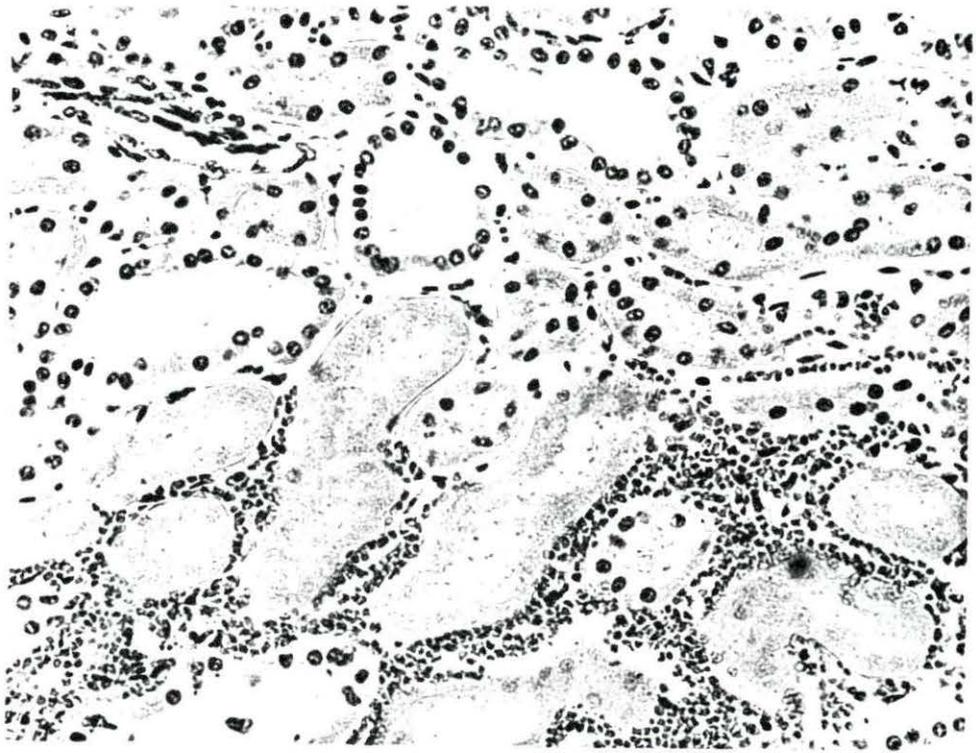
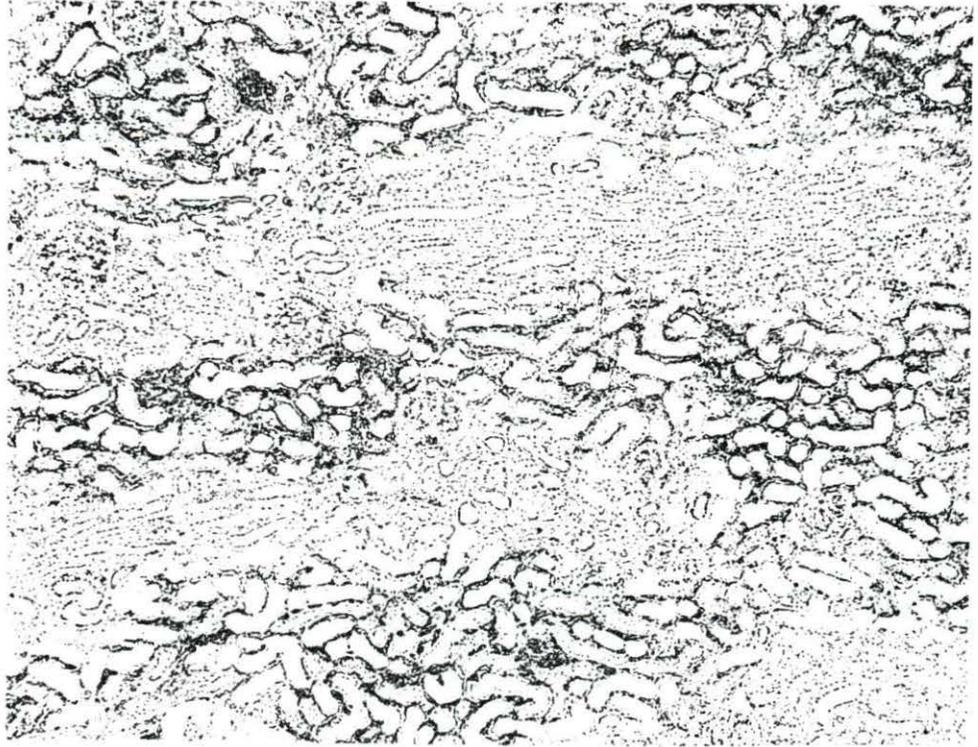
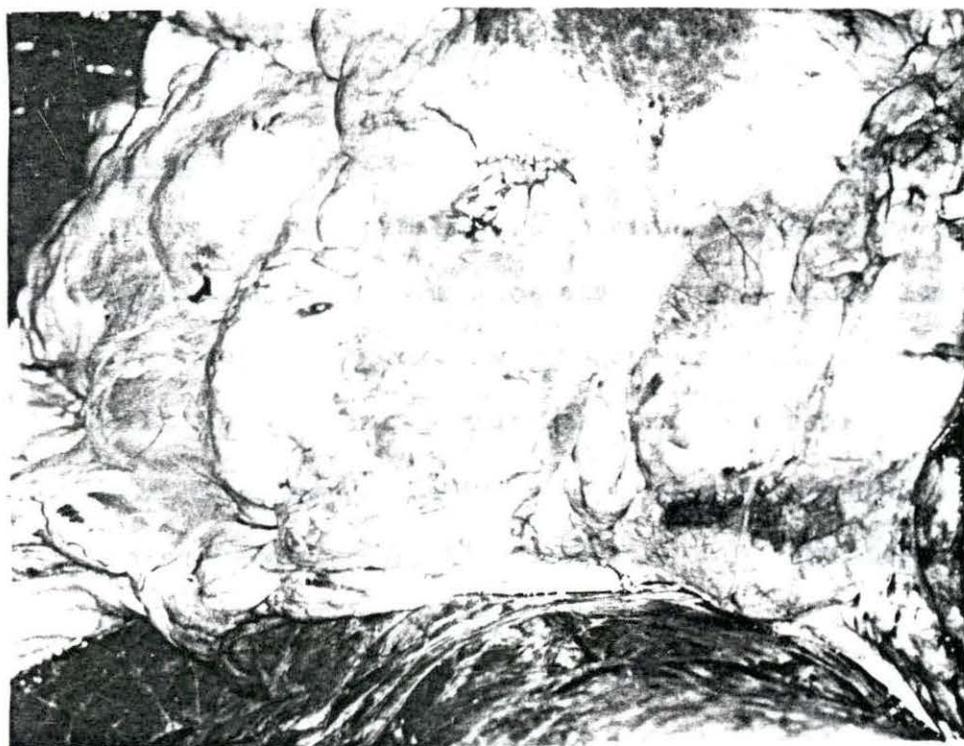


Fig. 11. Diffuse pericardial emphysema, 15 days after initial exposure to nitrogen dioxide by inhalation



Microscopically there was diffuse swelling and multiple foci of coagulative necrosis in myocardial sections from two animals. Edema of the vessel walls (Fig. 12), mild muscle hypertrophy and many thrombi were found in the pulmonary arteries. Thrombosis and hyaline (fibrinoid) degeneration of several vessels were present in one kidney (Fig. 13).

Digestive system Gross examination revealed general passive hyperemia. The livers of most animals were softer and of less intense color than normal. The abomasum of one contained several small ulcers and the omasum of another was approximately twice normal size.

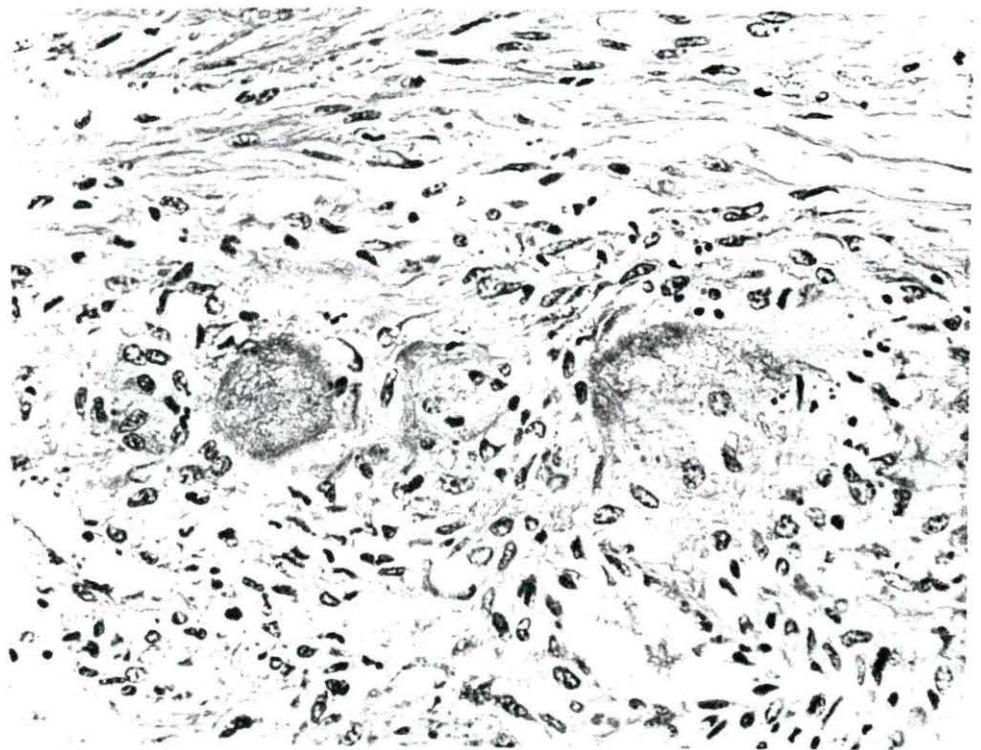
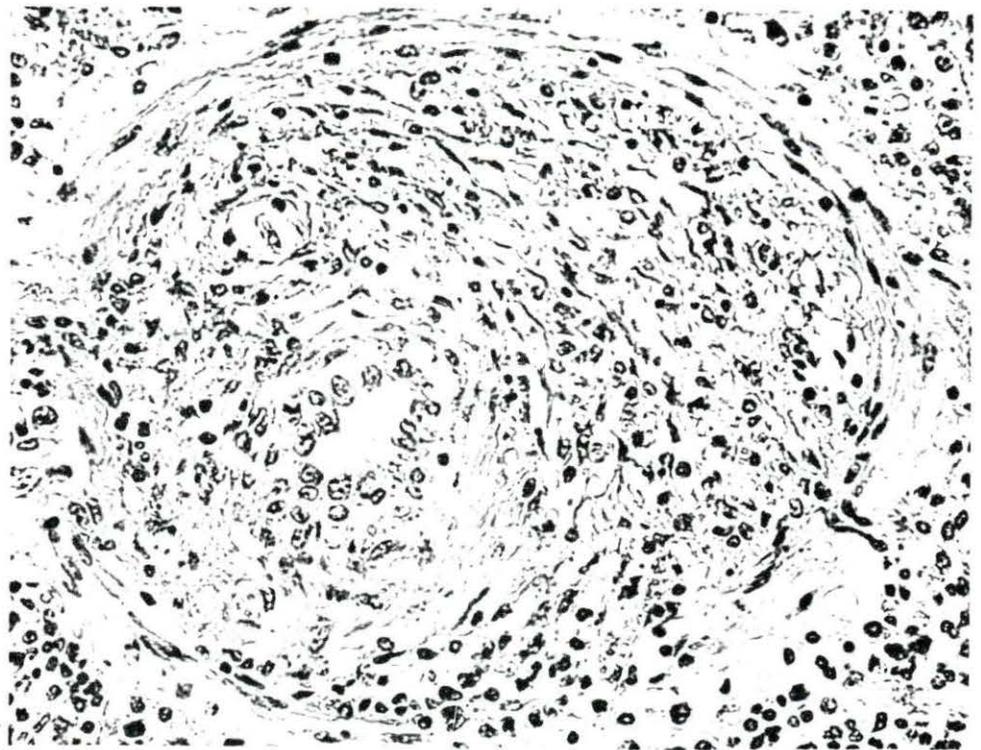
Microscopically the passive hyperemia was seen to be especially prominent in the intestines and the pancreatic and salivary glands. There was mild cloudy swelling and fatty degeneration of the hepatic parenchyma. Glycogen was present in very small quantities in the centrilobular regions of four of the six animals.

Endocrine system Mild to severe passive hyperemia of the pituitary, thyroid, pancreatic and adrenal glands in most cases, and hemorrhage in the thyroid glands of one animal were the only changes observed.

Lymphatic system Gross examination of the lymphoid tissue draining the respiratory tracts revealed several alterations. The lymph nodes, tonsils and other lymphoid tissue of the pharyngeal and retropharyngeal regions appeared hyperplastic. Hemorrhagic foci were present in the cervical, bronchial, mediastinal and prescapular lymph nodes as well as thymic and splenic tissues of some animals. Because of distension with gas the bronchial and mediastinal lymph nodes of all

Fig. 12. Edema of pulmonary arteriole following inhalation of nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 250

Fig. 13. Hyalin (fibrinoid) degeneration of renal vessels following eight endotracheal exposures to nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 320



six were three to four times normal size. The cervical nodes of one animal were slightly emphysematous.

Microscopically, several lymph nodes contained multiple foci to diffuse accumulations of blood in the sinuses (Fig. 14). This was most severe in the cervical, prescapular and retropharyngeal nodes. The spleens of all, except one, also contained excessive accumulations of blood. Most of the lymph nodes were hyperemic. Lymphoid hyperplasia was slight and inconsistent in the lymph nodes draining the respiratory system and there was mild hyperplasia of the reticuloendothelial tissue of some nodes, especially the bronchial and mediastinal. Emphysema of the bronchial and mediastinal nodes was very extensive (Fig. 14).

Muscular system Gross examination of the skeletal muscles of all animals of this group, except one, revealed no lesions. The muscles of one contained multiple pale pink foci indicative of necrosis.

When examined microscopically the skeletal muscles of all six were seen to be diffusely swollen, with coagulative necrosis of numerous muscle fibers (Fig. 15).

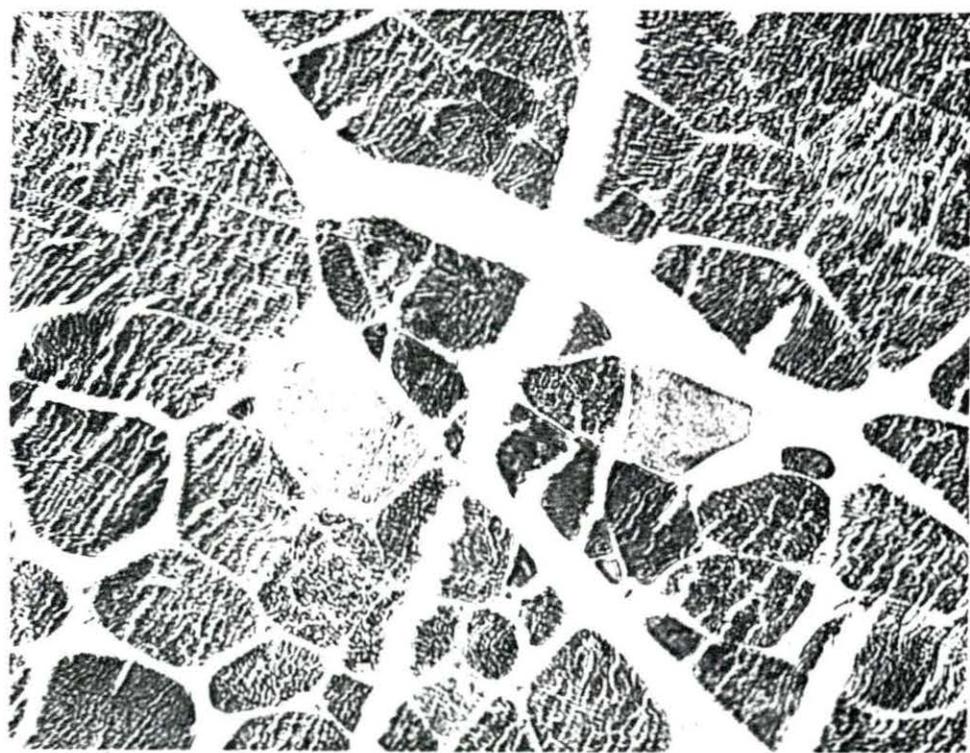
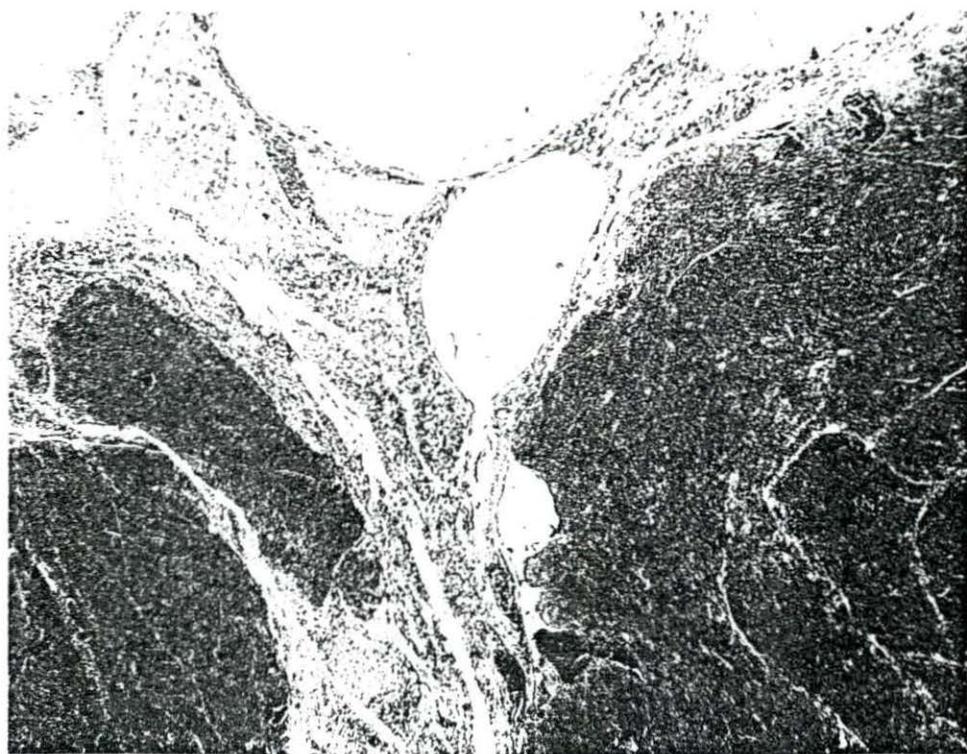
Nervous system No gross alterations were observed in the brains or spinal cords.

Microscopic examination revealed multiple small hemorrhages in many areas of the brains, spinal cords and meninges, and mild to moderate passive hyperemia.

Respiratory system Gross examination revealed major changes of the tracheas and lungs. Lymphoid hyperplasia, especially of the pharyngeal region, and mild hyperemia of the upper respiratory systems were noted.

Fig. 14. Mediastinal lymph node. Distention of sinusoids with blood and gas after exposure by inhalation  
Mayer's hematoxylin and eosin Y. x 20

Fig. 15. Skeletal muscle. Swelling and focal coagulative necrosis  
Gomori's one-step trichrome. x 350

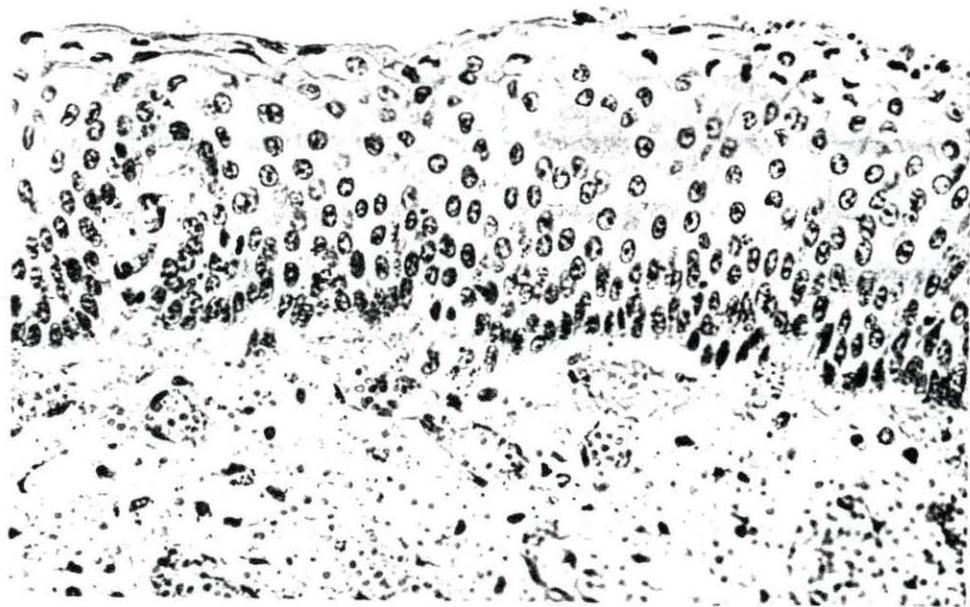


The tracheas, especially of those receiving nitrogen dioxide via tracheal tubes were very hemorrhagic and lined with fibrinous membranes. The trachea of most animals contained considerable frothy mucous and serous fluid.

Microscopic examination of the upper respiratory systems revealed similar changes in five of the six animals of this group. The exception was the one that died after only one exposure to gas. The upper respiratory tissues of this animal had minimal lesions, including a few submucosal hemorrhages, mild epithelial hyperplasia of the trachea at its bifurcation and mild general hyperemia. Examination of the turbinates, nasopharynges and larynges of the other five revealed mild active hyperemia, and hydropic changes in the epithelium. Mild squamous metaplasia of the epithelial tissues was also present in most instances. Focal accumulations of mononuclear cells and neutrophils were occasionally found in the submucosa. In addition, there was mild hyperplasia of the lymphoid tissue of the nasopharynges. In each of these five cases there was extensive coagulative necrosis of the epithelial and subepithelial tissues of the trachea, with a defense line of neutrophils and lymphocytes below the necrotic tissue and severe hemorrhage in the submucosa. Most of the epithelium had sloughed and the remaining tissue was covered by a thick layer of fibrin. Viable epithelium remaining was of the squamous type (Fig. 16).

Grossly the lungs were greatly enlarged, did not collapse upon opening the thoracic cavities, and several had imprints of the ribs on their surfaces. Most of the lungs were two to three times normal size.

Fig. 16. Trachea at bifurcation. Squamous metaplasia of epithelium  
14 days after initial exposure by inhalation  
Mayer's hematoxylin and eosin Y. x 320



Mottling was prominent due to lobules of consolidation alternating with lobules of alveolar emphysema. Consolidation was more advanced in the anterior lobes and emphysema more prominent in the posterior lobes. The consolidated lobules were dark red to bluish pink in color (Figs. 17, 18). Interstitial emphysema was massive and diffuse. Large interlobular gas filled cavities, up to 15 cm. in diameter in one instance, were present in the interstitial tissue (Fig. 19). Subpleural emphysema was prevalent in all of the lungs. The left diaphragmatic lobe of one animal was ruptured due to overdistension with gas. Emphysema of the mediastinum was severe with large gas accumulations up to 10 cm. in length and 5 cm. in diameter (Fig. 20). Slight to moderate emphysema of the connective tissue surrounding the trachea was present in all instances and in two the perirenal fat contained numerous gas bubbles. A few thrombi were found on cut surfaces of the lungs.

Microscopic alterations of the lungs of all six animals were quite similar. No consistent differences were noted between sections from different lobes or between different areas of individual lobes of the lungs. There was a definite lobular distribution of consolidated and emphysematous areas; usually more of the consolidated lobules were present. In the consolidated areas low cuboidal cells, characteristic of bovine pulmonary adenomatosis, were found lining many alveoli (Figs. 21, 22, 23, 24). These pavementing cells were not, however, present in all sections of lung examined but appeared randomly throughout the lungs. Large foamy macrophages were seen in many alveoli regardless of the presence or absence of lining cuboidal cells (Fig. 25). Multiple

Fig. 17. Greatly enlarged firm lung with multiple areas of consolidation and emphysema. Pericardial emphysema is also evident. Death occurred 15 days after first inhalation of nitrogen dioxide

Fig. 18. Close-up of lung with multiple consolidated and emphysematous lobules and subpleural emphysema. Death occurred 25 days after initial inhalation of nitrogen dioxide

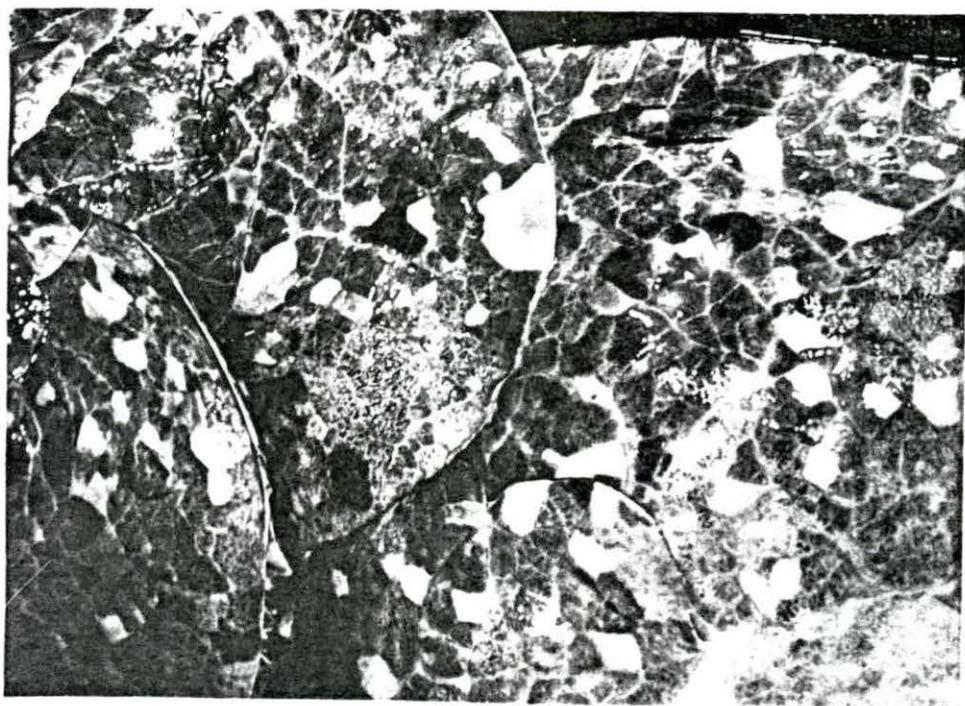
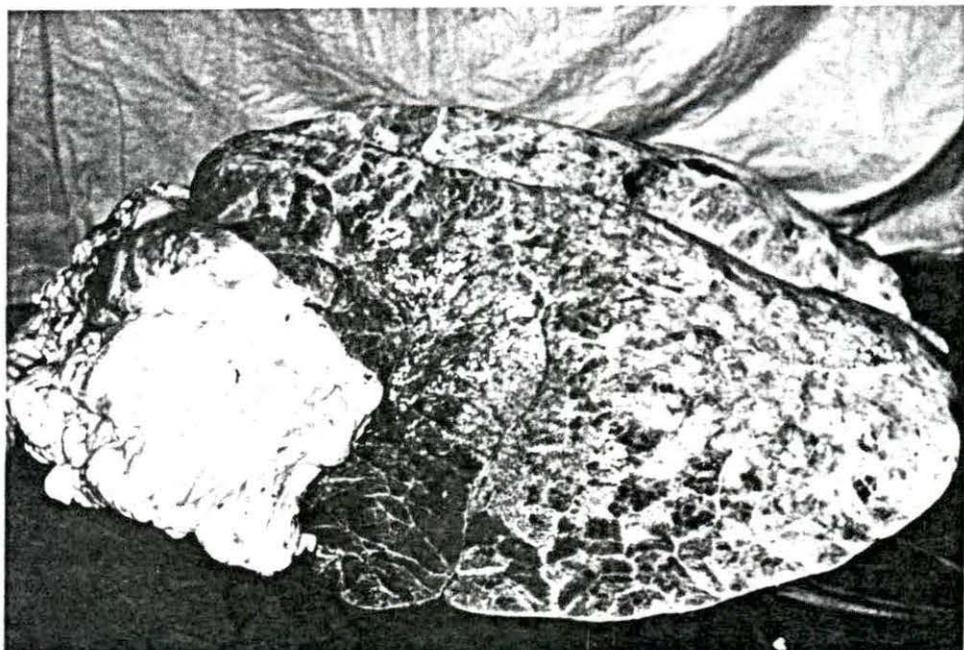


Fig. 19. Incision through a large gas filled cavity in intralobular tissue of the lung. Death occurred three days after initial inhalation of nitrogen dioxide

Fig. 20. Lung of same animal as Fig. 19 with distention of the mediastinum by gas

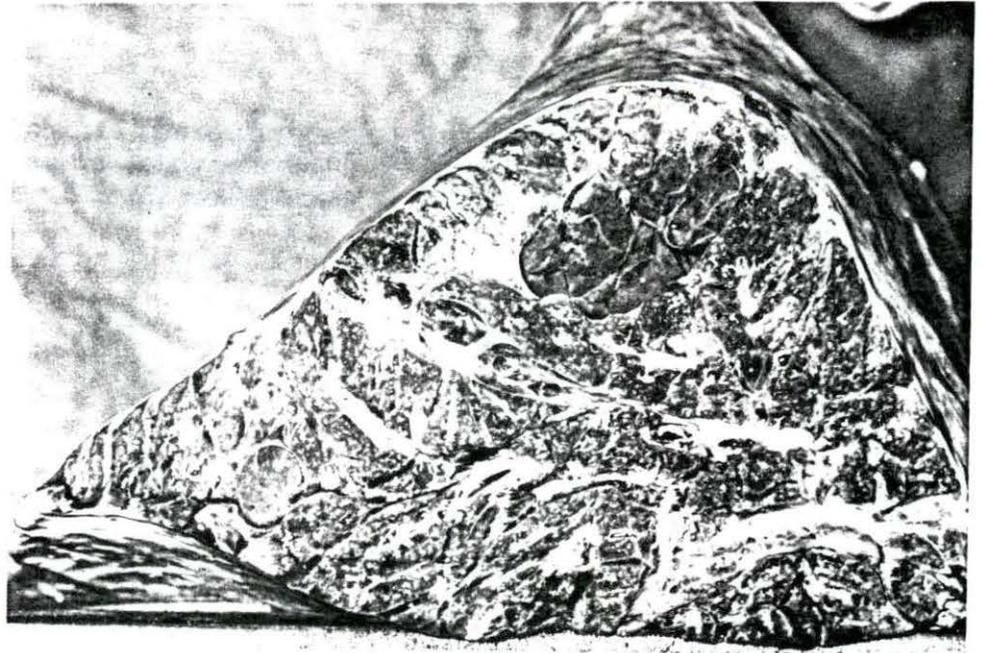


Fig. 21. Lung with proliferated alveolar epithelium lining the alveoli, forming a gland-like (adenomatous) appearance. Section from animal dying 25 days after initial inhalation of nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 320

Fig. 22. Higher magnification of Fig. 21  
x 500

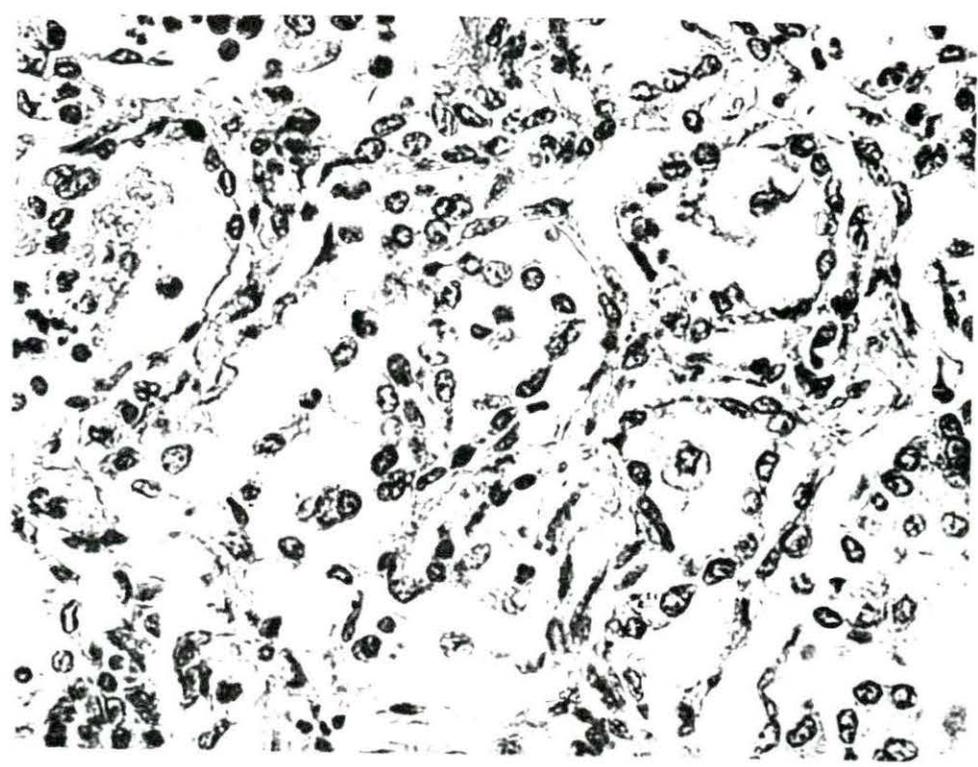
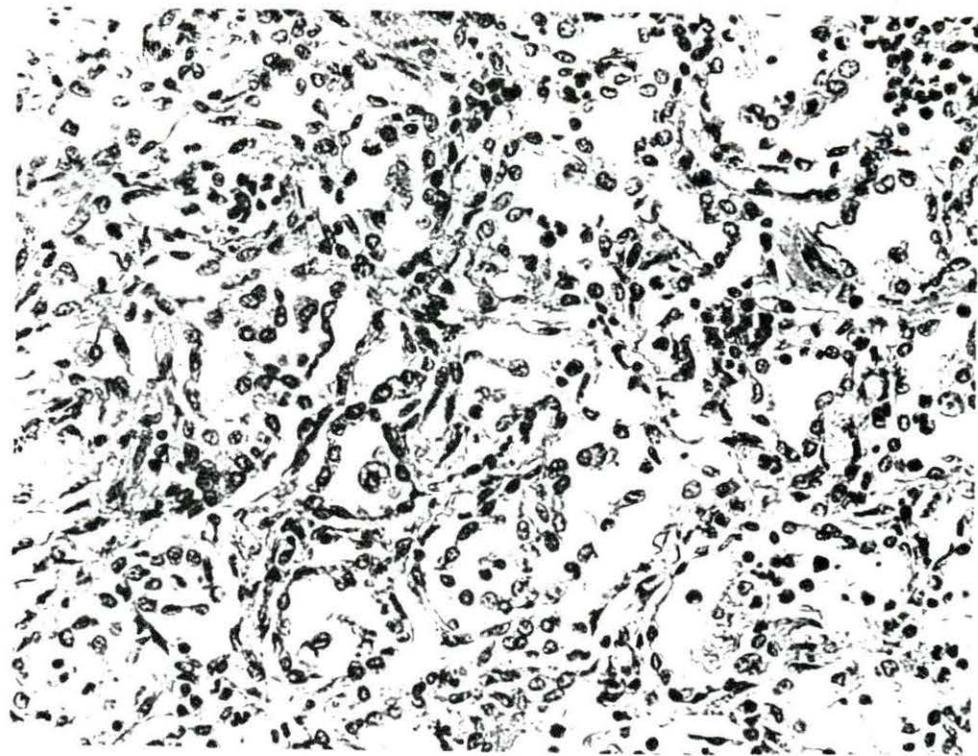
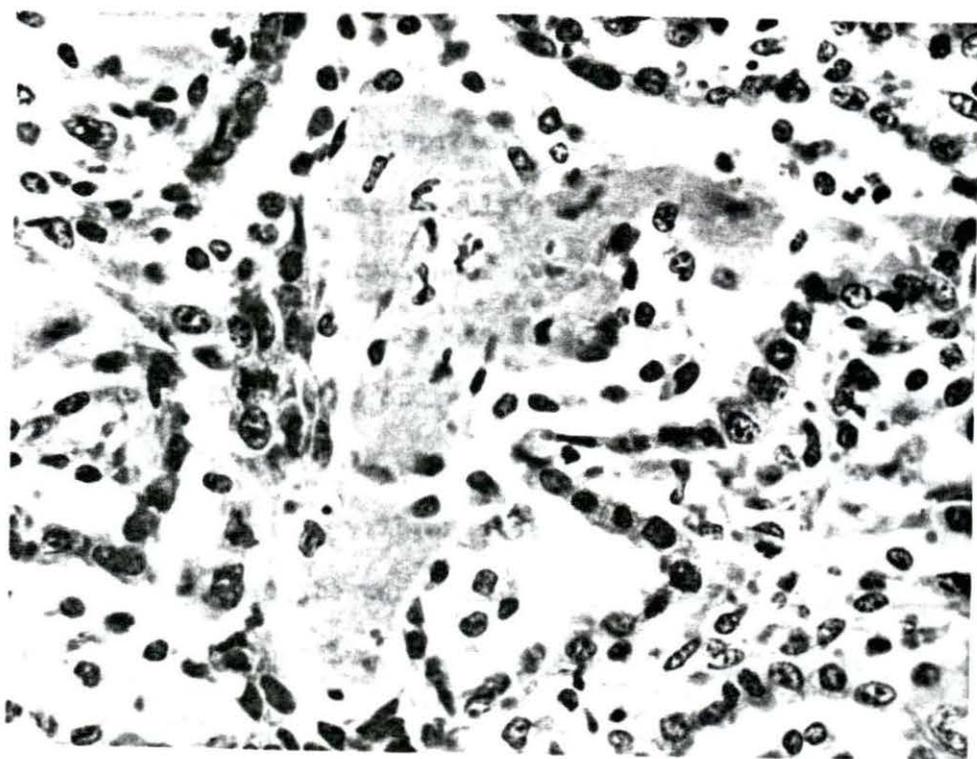


Fig. 23. Adenomatous epithelial proliferation of alveoli containing fibrin. From an animal inhaling nitrogen dioxide eight times, death occurring four days later  
Mayer's hematoxylin and eosin Y. x 125

Fig. 24. Higher magnification of Fig. 23  
x 500



necrotic foci of infarction were present in all lungs and thrombosed vessels were frequently found in and around the infarcts (Fig. 26). The alveoli of these infarcted areas were filled with fibrin and blood (Fig. 27). Hemorrhage and fibrin were also present in the lumens of many viable alveoli where the fibrin was frequently found as a lining hyaline membrane (Fig. 28). In addition to the alveoli, a few bronchi and bronchioles were filled with or contained deposits of fibrin (Fig. 26). Active hyperemia was severe in all lungs, the septal capillaries and arteries being engorged with blood (Fig. 29). Pulmonary edema was present in all animals but was severe in only two of the six (Fig. 29). Small atelectatic areas were occasionally seen. Prominent alveolar and interstitial emphysema was seen in the lungs of all six animals (Fig. 30). Alveolar emphysema was confined primarily to individual lobules interspersed among consolidated areas. Extensive hyperplasia and, in many instances, squamous metaplasia, were observed in the bronchial and bronchiolar epithelium (Figs. 31, 32). Hypertrophy of this epithelium was prominent in only one animal. Chronic bronchitis and bronchiolitis with proliferation of subepithelial connective tissue of numerous bronchi and bronchioles was found (Figs. 26, 33). This proliferation was sufficient to occlude many bronchioles, especially in the more chronic cases (Figs. 34, 35). The pulmonary musculature was slightly hypertrophied. The peribronchial lymphoid tissue was hyperplastic in all animals and the peribronchial glands in one was moderately hyperplastic. Small suppurative foci were found in the pulmonary parenchyma of several sections.

Fig. 25. Large foamy macrophages filling an alveolus  
Mayer's hematoxylin and eosin Y. x 500

Fig. 26. Thrombus adjacent to a bronchiole with chronic proliferative  
inflammation. Fibrin is present in the lumen. Death occurred  
12 days after initial inhalation of nitrogen dioxide  
Weigert's fibrin stain. x 100

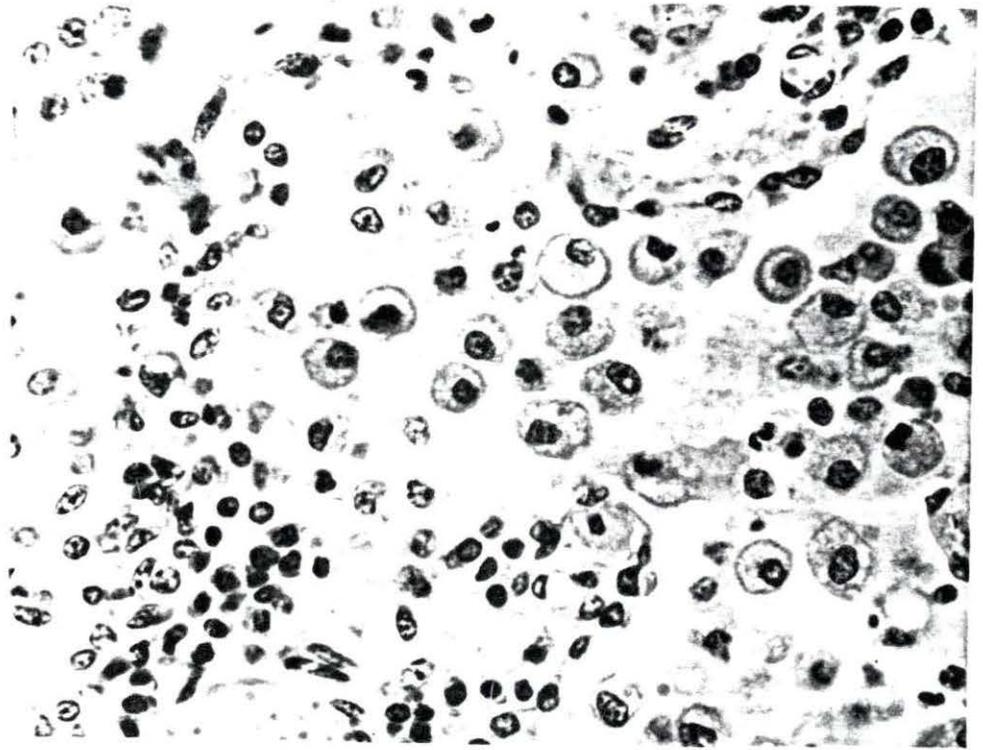


Fig. 27. Fibrin deposition, hemorrhage and hyperemia of an infarcted area of the lung  
Weigert's fibrin stain. x 100

Fig. 28. Fibrin lining alveoli in the form of a hyaline membrane  
Mayer's hematoxylin and eosin Y. x 320

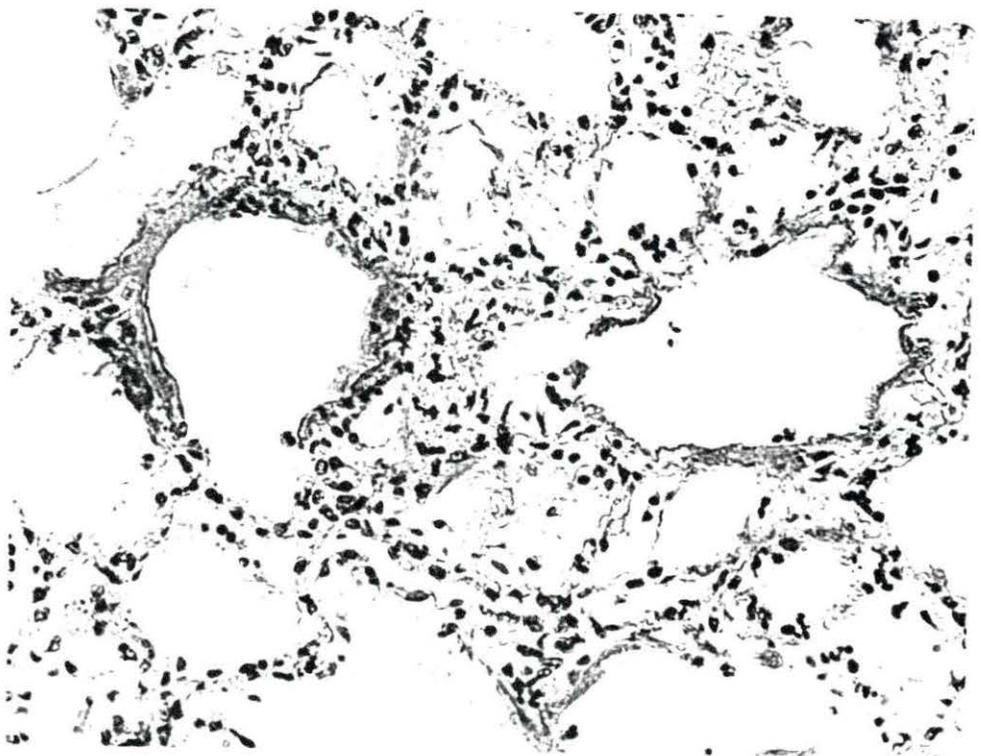
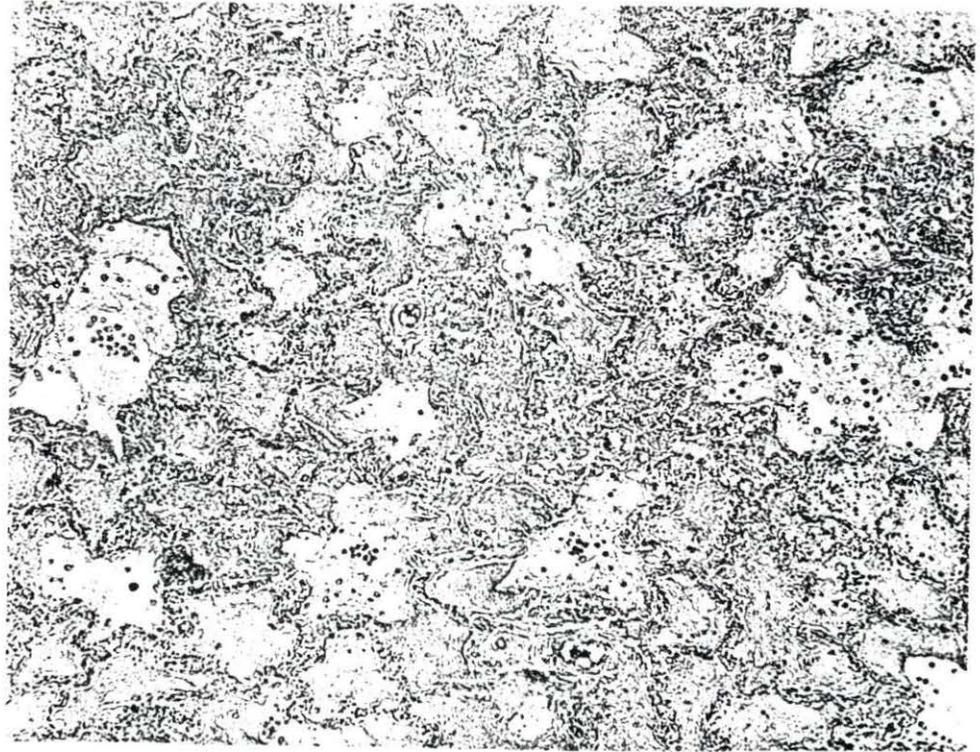


Fig. 29. Pulmonary edema and hyperemia.  
Mayer's hematoxylin and eosin Y. x 125

Fig. 30. Alveolar and interstitial emphysema adjacent to an infarcted  
lobule  
Mayer's hematoxylin and eosin Y. x 20

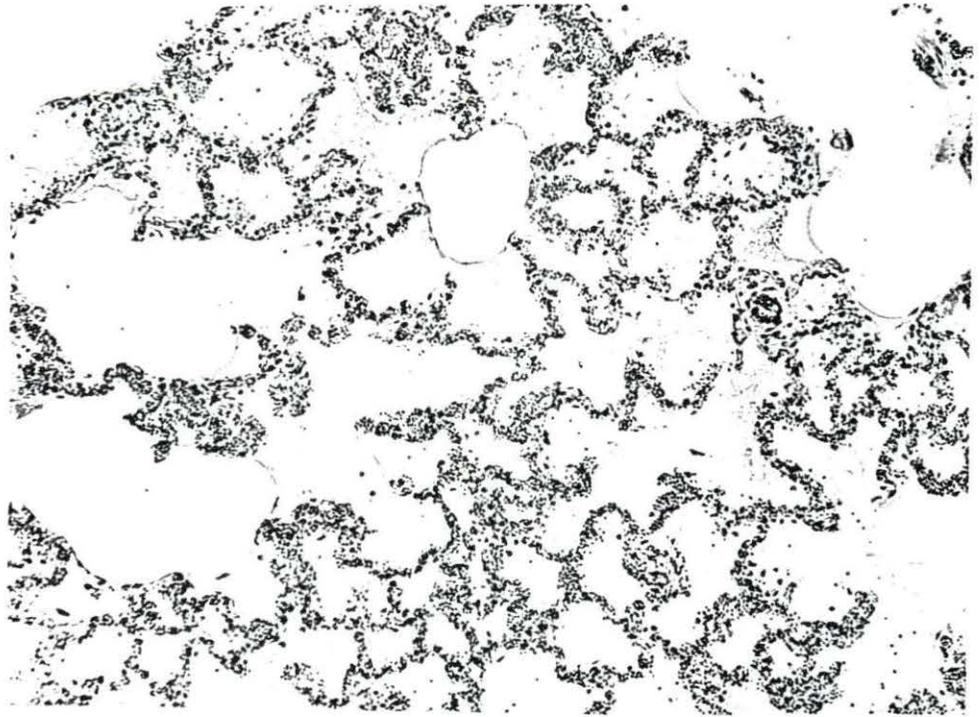


Fig. 31. Hyperplasia of bronchiolar epithelium 12 days after first  
inhaling nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 320

Fig. 32. Squamous metaplasia of a large bronchiole following inhalation  
of nitrogen dioxide. Animal died 14 days after initial  
exposure  
Mayer's hematoxylin and eosin Y. x 100

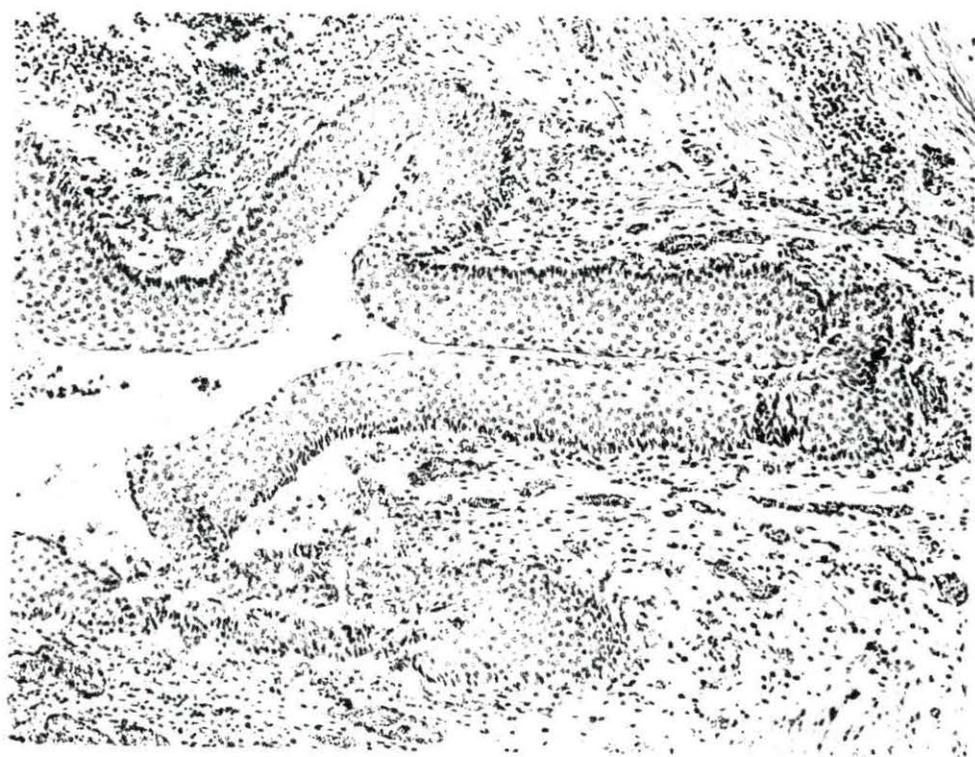
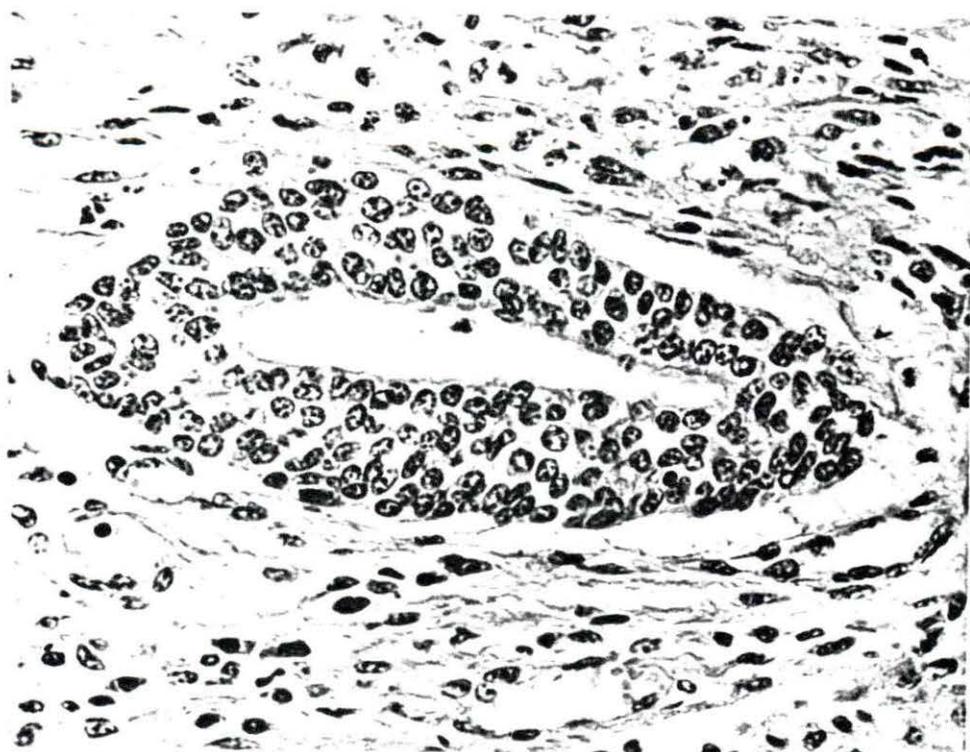


Fig. 33. Chronic proliferative bronchiolitis (bronchiolitis fibrosa proliferans) causing partial occlusion of bronchiole. Death occurred 12 days after initial exposure to nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 125

Fig. 34. Similar to Fig. 33 with more advanced fibrosis and occlusion of bronchiolar lumen  
Mayer's hematoxylin and eosin Y. x 100

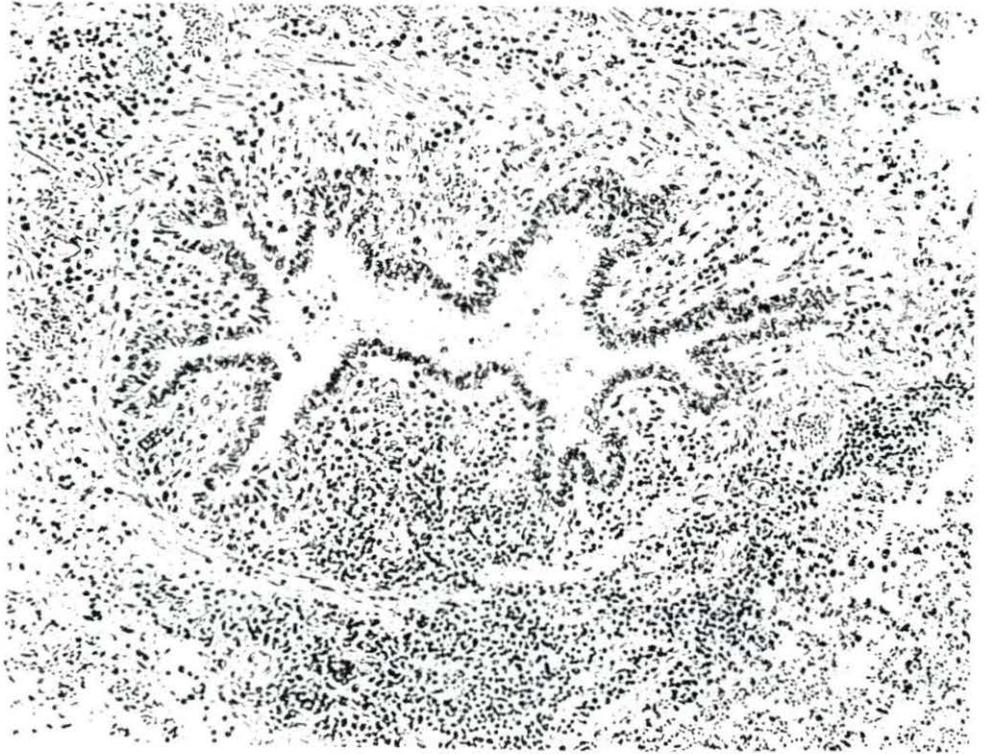
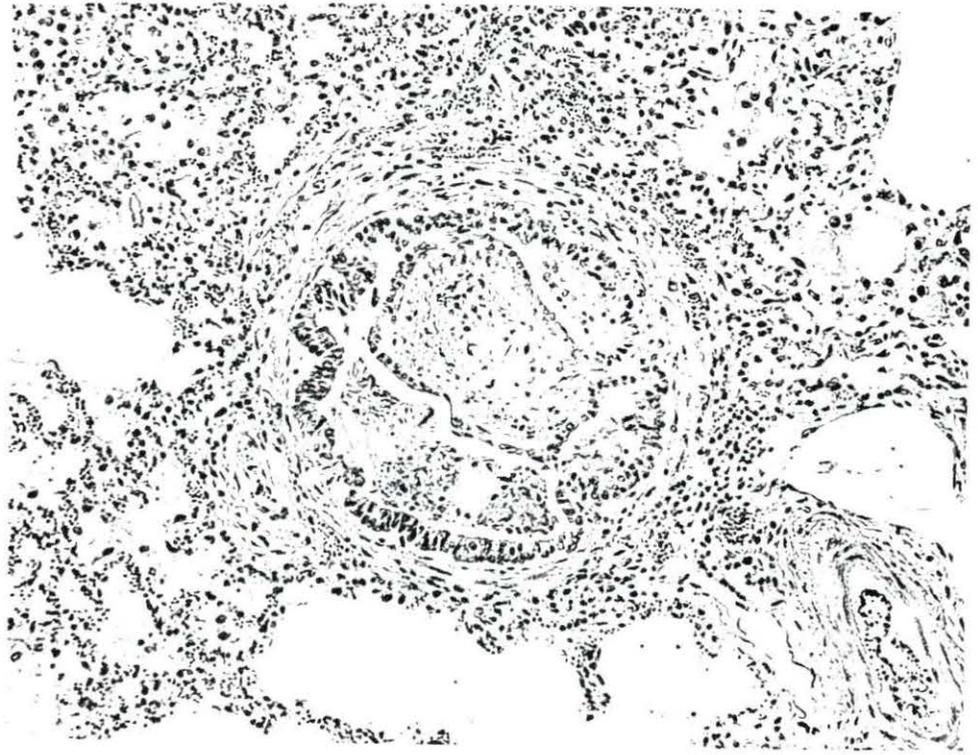


Fig. 35. Polypoid fibrous projection extending into the lumen of a small bronchiole. Death occurred 14 days after initial inhalation of nitrogen dioxide  
Mayer's hematoxylin and eosin Y. x 125



11

Urogenital system      Degenerative changes of the kidneys, other than mild hyperemia, were the only gross or microscopic alterations observed. Grossly the surface of the kidneys varied in color from diffuse pink to dark red or a mottling of these colors. The areas of discoloration usually extended into the medulla.

Microscopic study of the kidneys revealed many variable sized foci of severe swelling, coagulative necrosis and sloughing of the epithelium of the proximal convoluted tubules similar to that seen in the intraruminally exposed group (Figs. 12, 13). A large infarct, with thrombi, was present in one section. The necrotic tissue was surrounded by a zone of hemorrhage and hyperemia. Mild to moderate hyperemia was seen in the urinary bladders, uteri, and ovaries.

Lesions following exposure to nitric acid vapor

Cardiovascular system      No gross or microscopic lesions were observed.

Digestive system      The only consistent change found in the digestive tracts was a mild passive hyperemia of the small intestines and the parotid salivary glands. Several foci of hydropic degeneration of the rumen, reticulum and omasum were present in one animal.

Endocrine system      No gross or microscopic lesions were found in the pituitary, thyroid, or adrenal glands.

Lymphatic system      No consistent lesions were found in either animal. Microscopic examination revealed reticuloendothelial cell hyperplasia in several lymph nodes of one animal and in only a few nodes of the other. The splenic sinusoids of one were distended with blood.

Muscular system No gross lesions were observed. Microscopic examination of the pectoral muscles revealed diffuse swelling and multiple foci of coagulative necrosis. The same lesions were seen in the lingual muscles.

Nervous system Gross examination revealed no lesions. There were multiple petechiae in most areas of the brains and spinal cords as seen in sections.

Respiratory system Gross examination of the upper respiratory tracts and lungs revealed no lesions except slight alveolar emphysema of the antero-ventral portions of the lungs.

Microscopically there were focal areas of hydropic degeneration and mild hyperplasia of the epithelium, as well as subepithelial infiltration by lymphocytes, of the tracheas, larynges, and nasopharynges. Mild submucosal edema and hemorrhage were also observed in the tracheas.

Microscopic examination of the lungs revealed active hyperemia, edema and a few focal hemorrhages. There was mild hypertrophy and hyperplasia of the bronchial and bronchiolar epithelium as well as hyperplasia of the peribronchial glands and lymphoid tissue. Mild alveolar and interstitial emphysema were present. No proliferation of alveolar epithelial cells was found.

Urogenital system No gross or microscopic lesions were observed in the uteri or ovaries. There was mild passive hyperemia of the kidneys of both animals.

## DISCUSSION

Nitrogen dioxide occurs in two molecular forms, the dioxide form ( $\text{NO}_2$ ) and its dimer, dinitrogen tetroxide ( $\text{N}_2\text{O}_4$ ). These oxides are in a strongly temperature dependent equilibrium (3). At  $40^\circ\text{C}$ . approximately 30 per cent is in the dioxide form and 70 per cent is in the tetroxide form (9).

According to Grayson (9) toxicity to the respiratory system results from formation of nitric and nitrous acids. As the gas is inhaled, the equilibrium consistent with body temperature is established. Both forms of gas react with water, on the epithelium, to form the toxic acids. Nitric and nitrous acids are produced from the tetroxide; whereas, the dioxide yields nitric acid and nitric oxide. The latter, in the presence of oxygen, is converted to the dioxide. Nitric and nitrous acids react with alkali salts in the respiratory tract to form nitrates and nitrites and in so doing cause irritation. The nitrites are absorbed and convert hemoglobin to methemoglobin.

This mechanism adequately explains some lesions of nitrogen dioxide poisoning found in this study, but others are obscure. Pulmonary lesions, such as edema, epithelial hypertrophy and hyperplasia, proliferative bronchitis and bronchiolitis and emphysema are undoubtedly initiated by this irritation. The ruminal lesion is no doubt produced in a similar manner. Many other lesions apparently result from a basic vascular change, the origin of which is uncertain. It may be attributed either to hypoxia, the result of methemoglobinemia or to a more direct

action of the gas. The latter seems more plausible because of the lack of neuronal alterations, which should have occurred if hypoxia was sufficient to account for the vascular damage observed.

Although this project was not designed to study the pathogenesis of nitrogen dioxide poisoning, the sequence of changes in the lungs appears similar to those suggested by Lowry and Schuman (10, 11) in silo filler's disease. They postulated a continuous spectrum of conditions which are dependent upon the concentration of gas and duration of exposure. With very high concentrations the change is a fatal pulmonary edema. With a lower concentration there is less edema but extensive bronchopneumonia resulting in chronic obliterative bronchiolitis which is fatal in three to five weeks. Exposure to still lower concentration causes a nonfatal bronchiolitis with focal pneumonitis. These same stages were seen in our cattle and corresponded well with duration of exposure. They did not list emphysema as a major lesion of silo filler's disease. The emphysema in cattle probably results from violent respiratory efforts secondary to pulmonary edema, fibrin deposition and proliferative changes; therefore is not specific for any stage.

Lesions of pulmonary adenomatosis, among others, are easily induced in cattle by inhalation of nitrogen dioxide. This is not true for intraruminal administration of the toxic gas. Although minimal lesions indicative of adenomatosis are produced by rumen insufflation, the degree of severity and characteristic alveolar epithelial proliferation do not

develop. Thus, the proposal that nitrogen dioxide of ruminal origin causes clinical adenomatosis seems questionable.

Other factors also indicate that nitrogen dioxide probably is not the cause of this disease. Some lesions of experimental nitrogen dioxide poisoning are not seen in field cases of adenomatosis. The infarcts and fibrin deposition in the lungs, the diffuse necrosis of proximal convoluted tubules and infarcts of the kidneys as well as other vascular changes have not been reported in natural adenomatosis. The finding that only minor pulmonary lesions developed in intraruminally exposed animals as compared to those exposed by inhalation, yet methemoglobin levels were similar, suggests that death would result from anoxia prior to entrance of sufficient rumen origin gas into the lungs to cause adenomatosis. Also, decomposition of nitrogen dioxide in the presence of water would prevent its eructation and inhalation.

Even though lesions of adenomatosis were not induced by rumen insufflation, it cannot be positively stated that nitrogen dioxide is not involved with the disease. Differences in experimental exposure and that proposed for the naturally occurring condition definitely occurred. The most significant of these was the daily administration of gas as compared to an expected continuous production of gas in natural cases. Perhaps continuous intraruminal exposure to a small amount of gas would be successful in producing more definite pulmonary damage.

Although nitric acid vapor has been reported (13) to cause considerable pulmonary damage in man, only minimal changes were produced in our cattle. Because of lack of information regarding dosage,

both time and concentration were probably insufficient. Possibly an increase in either or both would induce lesions similar to those of nitrogen dioxide poisoning.

Many chemical irritants as well as bacterial and viral agents are capable of causing alveolar epithelial hyperplasia. Bell (2) described a number of these conditions, including chronic passive congestion of the lungs, lipoid pneumonia and chronic interstitial pneumonia. He cited other reports and concluded that any disease which brings about "marked" thickening of the interalveolar septa, with displacement of the capillaries away from the surface, or a mild irritation of the alveolar walls with foreign material, may result in "epithelization." Omar (18, 19) has assembled an extensive review of the literature concerning alveolar epithelial hyperplasia and conditions in which it is found. Spencer (24) concluded, from comparative pathology and from investigation of the pathogenesis of alveolar cell hyperplasia in man, that it is related to chronic pulmonary damage. Possibly inhaled irritants together with many other transient causes of lung damage are all equally capable of evoking the change.

Of the many irritants capable of causing pulmonary damage, several produce changes that are in many respects similar to the lesions of bovine pulmonary adenomatosis. Many of the war gases studied by Winternitz (28) when in low concentrations are examples. Included in this group are chlorine, phosgene ( $\text{COCl}_2$ ), diphosgene or superpalite ( $\text{ClCOOCCl}_3$ ), and chloropricrin ( $\text{CCl}_3\text{NO}_2$ ). Upon inhalation all caused severe pulmonary edema and epithelial injury as well as varying degrees

of emphysema, atelectasis and chronic obliterative bronchiolitis.

Other chemical irritants with varied lesion-producing capabilities are: mercury vapor (25), nitric and sulfuric acids (13), hydrochloric acid (16, 29), ethyl alcohol (17), cadmium oxide and ammonia (24).

The nonspecific nature of the lesions of bovine pulmonary adenomatosis is illustrated by this variety of conditions and poisonings in which similar lesions occur. Thus, the etiologic agent could conceivably be one of several pulmonary irritants.

## SUMMARY

The proposal is made that nitrogen dioxide or other toxic gas of rumen origin is responsible for bovine pulmonary adenomatosis. The experimental method and results of exposing cattle to nitrogen dioxide by various routes and to nitric acid are described.

Daily inhalation of nitrogen dioxide resulted in methemoglobinemia, severe dyspnea and death. Pulmonary lesions consisted of hyperemia, edema, hemorrhage, fibrin deposition, hypertrophy and hyperplasia of bronchial and bronchiolar epithelium, alveolar epithelial proliferation, chronic proliferative bronchiolitis, alveolar and interstitial emphysema and infarction. Emphysema was also prominent in the mediastinum as well as the mediastinal and bronchial lymph nodes. Diffuse coagulative necrosis of proximal convoluted tubules and in certain instances multiple infarcts were found in the kidneys. In addition to multiple pulmonary and renal thrombi, edema and hyalinization of the walls of several vessels were present.

In contrast to inhalation of nitrogen dioxide, rumen insufflation resulted in minimal pulmonary lesions. The only fatalities resulted from methemoglobinemia. There was mild bronchial and bronchiolar epithelial hyperplasia, hyperemia, edema, atelectasis and emphysema. Coagulative necrosis of the proximal convoluted tubules was prominent. Ruminal necrosis was severe in the area where the gas was introduced. General passive hyperemia was present following both inhalation and intraruminal exposures.

Inhalation of nitric acid vapor induced only minor pulmonary epithelial lesions. Alveolar epithelial cell proliferation did not result from either rumen insufflation of nitrogen dioxide or from inhalation of nitric acid vapor.

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