Atmospheric composition in an enclosed swine production building

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ATMOSPHERIC COMPOSITION IN AN ENCLOSED SWINE PRODUCTION BUILDING

by

James Anthony Merkel

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

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INTRODUCTION

The quality of the atmosphere, has been recognized as an important variable in the environment only during the past few decades. Today, we can no longer take the air we breathe for granted.

As a result of industrial growth and urban development, the air around us is becoming increasingly polluted with undesirable gases. Recently, we have been alerted by severe outbreaks of illnesses caused by air pollution. In addition to these more serious problems of public health, we now are confronted with many other troublesome manifestations of air pollution; such as, plant damage, reduced visibility, odors, and economic blight. As agriculture continues its trend toward modernization and large scale production, farmers are becoming aware of their own contributions to air pollution and the implications contaminants may have on their operations.

Today large numbers of animals are housed in confined areas where attempts are made to provide optimum conditions for their productivity. Until recently, however, most of the research into livestock environment has been devoted to studying the effects of temperature, humidity, and ventilation on the growth rate, feed efficiency, and productivity of the animals. Comparatively little information is available on the effects of variation in the composition of the atmosphere on growth and production or even on the extent to which such variations may occur.

Even the most simple shelter results in a reduction of mean air velocity and it is in this respect that indoor climates differ most from outdoor climates. The reduction of natural air movement indoors and restriction of the air space allotted to each animal create an undesirable environment as noxious gases and water vapor accumulate.
In 1965, the giant Monfort Feed Lots of Greeley, Colorado, were faced with a $300,000.00 damage suit by a neighboring couple as a result of obnoxious feedlot odors (2). The Roy F. Benton Feed Yard in Pomona, California (2) also was brought to court on a public nuisance suit and was forced to initiate an odor control program set up by the courts.

Cattle feeders in Arizona have been faced also with the problems of air pollution (3). Citizens of Tempe, Arizona, filed suits in 1963 asking for $859,000.00 in damages against four cattle feeding companies operating within the city limits. These suits contended that cattle feeding cause "vile, striking, obnoxious, and nauseating odors".

Most of these odor problems are a result of the large commercial, western feedlots where cattle populations vary from 3,500 to 50,000 head during the peak season. The average mid-western farmer, on the other hand, is currently feeding around 100 head of cattle and 300 to 500 head of hogs. Estimates from the Iowa State Animal Science Extension Service, however, indicate a trend toward bigger feeding operations. Today in Iowa there are over 1000 cattle-feeders and 1000 hog-feeders who raise more than 1000 head in their operation. The trend to larger feeding operations along with the increase in urbanization will undoubtedly bring about the advent of additional complaints and law suits concerning obnoxious odors and air pollution.

Besides the nuisance problem, however, cognizance must be taken of the economic implications that these gases may have on the overall productivity of the animals and the performance of the building over a period of time. Day et al. (4)

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The gases from fresh livestock excreta as well as those from body processes cause air pollution when sufficiently concentrated. Similarly, the bacterial decomposition of excreta, collected and stored with or without bedding material, also results in the release of a number of noxious gases.

The chemical quality and quantity of these gases in enclosed livestock buildings is virtually unknown. However, there is ample evidence that several of the gases released during the decomposition of excreta, if concentrated, can cause injury or even death to livestock. Prolonged exposure to low levels of these gases may be of considerable importance, although again, health effects are largely unknown.

Air pollutants also can have a damaging effect on building materials and equipment items. In the United States alone, a detailed study by Gibson (1) estimates that the economic loss on building material caused by air pollution from all sources exceeds 2 billion dollars per year.

The gases existing in a confined livestock building also become objectionable for the working personnel; low concentrations are irritating to the eyes and to the mucous membranes of the respiratory tract. In many instances, surrounding neighbors complain about livestock odors claiming them to be public nuisances.

The problems, then, created by the accumulation of gases in livestock buildings can be summarized as: (1) posing a threat to the health of an animal and impairing its productivity; (2) causing excessive deterioration of equipment and building materials; and (3) creating objectionable odors for farm personnel and nearby neighbors.

Currently, perhaps, the most pressing of these problems are objectionable odors which create nuisance effects to the farmstead as well as to adjoining neighbors. The importance of such happenings can be borne out by numerous court cases reported in the past few years and the ever-increasing number of complaints from nearby citizens.
report an unexplained decrease in the rate of gain of pigs around 150 pounds when the animals are raised in confinement buildings where wastes were ponded under the building floor for a month or longer. In Europe, (5, 6), many cases of animal deaths have been attributed to large quantities of toxic gases released from manure holding pits when agitated, such as takes place at the time of emptying the tanks. As complete confinement of animals becomes more widespread, we will also need to know what effect such odors can have on animals over a prolonged period as might be the case with breeding stock.

The more relevant characteristics of a large number of fumes, gases, and solid particles involved with a multitude of basic processes (including threshold limit values and toxicity to humans) can be found readily in a number of handbooks (7, 8). If farm animals, especially swine and poultry, are like humans in their reactions to these gases, then it is very probable that growth and production will be adversely affected when the animals are subjected to concentrations of the gases. The specific role that gases play in the overall performance of animals under confinement will become more important as we continue to increase efficiency in the livestock enterprise.

The primary purpose of this research project was to seek those gases present in a confined swine production system. Continued research undoubtedly will be required to describe the quantitative nature of the atmosphere under varying conditions. With this information, biochemists, physiologists, and metallurgists can better predict the effect individual contaminants have on animal and material performances. It also will enable engineers and scientists to more intelligently design environmental control systems for livestock building and to develop and employ techniques which will eliminate or control gases objectionable to the animals, the workers, materials, and the public.
AIR POLLUTION IN LIVESTOCK PRODUCTION SYSTEMS

Air pollution might be defined as the presence of substances in the ambient atmosphere put there by the activities of man or animal in concentrations that will interfere with comfort, safety, or health. However, an important distinction should be made between communitywide air pollution problems and localized problems. While there is a lot of overlapping of the two types, this distinction will help in considering the sources and effects of pollution and the type of control efforts required.

The localized problem is one of being able to describe what air pollutants exist in a completely confined livestock environment. This problem of air pollution has been created by the current trend in livestock production where large numbers of animals are housed in completely enclosed buildings with attempts to create optimum conditions for their productivity. Until recently most of the research concerning an animal's environment has been related to the effects of temperature, relative humidity, and ventilation rates on the performance of the animal. Very little attention has been given to the composition of the air surrounding the animal or what effects it has on the animal's performance or on building materials.

Several reports have indicated that, in some cases, confining livestock in totally enclosed buildings has resulted in decreased rate of gains, increased respiratory problems, poisoning from toxic gases, and asphyxiation. Several workers (9), (10), (11), investigating this area have reported information on the composition of the air above the floor in piggeries, generally in slatted-floor houses. Most of their work deals with the levels of ammonia, carbon dioxide, hydrogen sulfide, and methane.
Ammonia has been blamed for pig deaths in Norway (12) and Northern Ireland (13) by condensing on the walls and oxidizing to nitrites and nitrates. If accessible and taken internally, these accumulations may be fatal.

Hydrogen sulfide, a potentially toxic gas has been accused for a number of animal deaths in Sweden (13) and Denmark (14). This gas is released from holding pits immediately after agitation begins and if allowed to escape directly to the animal's surroundings may quickly reach concentrations sufficiently high to cause death.

Carbon dioxide has also been found to increase to undesirable concentrations under conditions of poor ventilation (10) and during periods when holding pits are agitated. Carbon dioxide is not as dangerously toxic as similar concentrations of hydrogen sulfide (15). Both of these gases, because they are more dense than air, will accumulate directly above the holding pit. Consequently, animals lying on slatted floors above such holding pits can inhale excessively high concentrations of these gases.

The evidence presented thus far clearly indicates that several of the gases released during the decomposition of manure can cause injury and even death to livestock. There is also evidence that concentrations of ammonia, considerably below those which produce visible signs of injury, can increase the susceptibility of poultry to virus infection (16).

Perhaps of more importance, is what effect prolonged exposure to low concentrations of these toxic gases will have on the health of the animals. Exposure to sublethal concentrations for short periods, such as when slurry below slats is disturbed, may also affect the health of animals.
When an effort is made to specifically analyze the hazard that air pollution creates for animal health, only fragmentary information is available, which makes it difficult to arrive at a sound and scientifically valid conclusion.

Knowledge in the field is steadily increasing, however, and there are a few general observations that appear to have validity with respect to air pollutants and their relationship to health. From the experiences in the Belgian Meuse Valley in 1930; in Donora, Pennsylvania in 1948; and in London in 1948, 1952, and 1956, under certain atmospheric conditions the pollutants in the air arising from home and industrial wastes reached lethal concentrations (17). In Donora, 20 deaths were attributed to one episode, with nonfatal illness affecting half the population. In London during 1952, between 4,000 and 5,000 additional deaths were experienced during one week of the episode.

Although most of the persons who died in these acute episodes were elderly and had histories of cardiac or respiratory ailments, there is clear evidence that extended exposure to certain types of air pollutants, can aggravate respiratory disorders.

The high incidences of "chronic bronchitis" in British cities, of nasopharyngeal and eye irritation in Los Angeles, and the rapid rise in lung carcinoma among metropolitan populations appear to be closely associated with air pollution (18).

Although very limited information is available showing what effect air pollution has on animals, much the same effect might be expected because both have very similar respiratory systems.

A cursory investigation of the Poza Rica episode, 1952 (19), disclosed that the animal population had been affected, in that an undetermined number of canaries, chickens, cattle, pigs, geese, ducks, and dogs either died or were made ill by the exposure. It was estimated that 50% of the exposed species (other than canaries) died from the air pollution.
Donora, Poza Rica, London, and the Meuse Valley of Belgium have dramatized that air pollution can kill; but even more, these experiences point out the extensive effects of air pollutants on the health of affected populations. Long-continued exposure to sublethal concentrations of many substances, and combinations thereof, are suspected of having physiological effects, but in most cases the qualitative aspects of the relationships remain undefined.

**Effects of Some Indigenously Produced Gases on Animals**

More subtle physiological effects of air pollution are suggested by findings of suppressed ciliary action, alterations in pulmonary physiology, specific enzymatic inhibitions, and changes in blood chemistry when laboratory animals are exposed to various gases which often are involved in pollution.

**Sulfur Dioxide**

Dalhamn (20) observed the influence of sulfur dioxide on mucous flow and ciliary activity of rats, and showed a considerably increased amount of mucous, and reddened mucosa of the trachea. The average exposure concentration of sulfur dioxide required to produce these changes approximated 11 ppm for 18 days. There was no change in ciliary beat until 25 ppm sulfur dioxide was obtained. Subsequent observation of the rats following sulfur dioxide exposure showed a considerably retarded flow of mucous; some animals showed no measurable mucous transportation, an important clearing mechanism of the respiratory tract. On the other hand, ciliary activity rapidly commenced following cessation of exposure, and the animals later showed no tolerance, "habituation", or hypersensitive reactions upon re-exposure.
Work by Amdur (21) showed greatly reduced breathing as sulfur dioxide concentrations increased from 26 ppm to 825 ppm. Disturbances in reflex activity and respiratory patterns have also recently appeared in the Russian literature (22). The lowest concentration of sulfur dioxide that produced reflex disturbance in the rabbit was between 1.2 and 3.7 ppm; changes in respiratory patterns occurred at 1 ppm.

**Ammonia**

Because of its high aqueous solubility, its physiological activity is confined almost entirely to the upper respiratory tract, only a few percent reaches the lungs in the inhaled concentration. At extremely high concentrations, ammonia acts as a powerful asphyxiating by constricting air passages, owing to its extreme irritancy. Although an irritant gas, the least amount producing immediate irritation of eyes, nose, and throat is from 400 to 700 ppm (23).

Ammonia is not believed to be a serious health menace in short intense exposures; but may have irritant or health impairing effects in prolonged repeated exposures. Weatherly (24) found that exposures of male guinea pigs to 170 ppm ammonia for 18 weeks, 6 hour/day, 5 days/week was required to produce mild changes in spleen, kidneys, adrenals, and liver, but that no changes were seen at 12 weeks.

In his remarks on the effects of ammonia as an air pollutant, Pottle (25) concluded that if the ammonia, as an air pollutant, could be beneficial by the partial neutralization of acetic airborne constituents such as sulfur dioxide, with consequent reduction in irritant activity of each constituent.
Carbon Monoxide

It is extremely doubtful that carbon monoxide will ever exert a serious effect on animal performance because attainment of injurious concentrations represent extremely large 8-hour additions of carbon monoxide to the atmosphere. Repeated daily exposures to 100 ppm of carbon monoxide did not produce ill-effects in humans (23). It does however, become fatal at slightly more than 10 times this level.

Formaldehyde and Homologues

Formaldehyde is recognized as a sensitizing agent and is highly irritating to the mucous membranes of the eyes, nose and throat. Unsaturated aliphatic aldehydes such as acrolein and crotonaldehyde are more highly irritating than formaldehyde. Kotin (26) exposed exteriorized rat and rabbit trachea and ciliated epithelium of the frog's esophagus to formaldehyde and a number of higher aldehydes, and found inhibition of ciliary action and of mucous flow immediately on exposure to 22 to 66 ppm. Acetaldehyde and n-butylaldehyde produced immediate stimulation at about 10 ppm, whereas propionaldehyde and isobutyraldehyde showed no immediate effect. 2-furfuraldehyde behaved like formaldehyde at first but later stimulated ciliary activity at somewhat higher concentrations. Interestingly, each aldehyde showed an individually characteristic response following exposures of 1 and 16 minutes and of 6 hours duration. Dalhamn (20), in testing concentrations of formaldehyde of 22, 10, 3 and 0.5 ppm on the ciliary movement of the respiratory tract of anaesthetized rats with opened trachea, found that movement ceased and thus cessation of mucous transport at 10, 30, 50 and 150 seconds respectively. Crolley (27) had previously shown similar effects in roughly the same interval of time on the excised trachea of rabbits, but does not report whether ciliary activity remained depressed for long periods after prolonged depression. Continued depression of ciliary activity permits
bacterial accumulation, which, if infectious, can lead to secondary complications in the respiratory tract.

The sensitizing action of formaldehyde (23) is well known but not documented with sufficient evidence to permit an estimate of what order of concentration is required to produce hypersensitivity. The lowest concentration giving detectable odor is said to be 0.8 ppm., and the lowest concentration causing throat irritation, 5 ppm.

**Organic Sulfur Compounds**

Until recently very little attention had been given the organic sulfur compounds in the air. Conflicting toxicity reports and the little available information on these substances prompted a detailed study of the acute toxicity of 7 aliphatic and 2 aromatic thiols in animals by Fairchild and Stokinger (28). The 9 compounds tested were benzene-, methylheptane-, -toluene-, ethane-, hexane-, butane-, propane-, 2-methyl-2-propane, and 2-methyl-1-propanethiol. Several routes of administration were used, including that of inhalation and exposure. Of the 7 compounds tested by inhalation in rats, rabbits, and mice, benzenethiol and methylheptanethiol were the most toxic.

All thiols had the common toxicological property of being saparific, and ranging in degree from producing mild stupor to heavy sedation. Methylheptanethiol was an exception in that it produced a powerful central nervous system stimulation.

Acute thiol poisoning produced a uniform pattern of central depression and respiratory paralysis, death ensuing from respiratory failure.

The most toxic thiols when administered 3 times each week were tolerated by rats without indication of either cumulative toxicity or increasing level of tolerance (23).
Thiol exposed rats and mice commonly showed latent pulmonary infection and/or pneumonia.

Meager quantitative toxicity information is available on the organic nonthiol sulfur compounds, the sulfides and the disulfides. Comparative information shows dimethyl sulfide \((\text{CH}_3)_2\text{S}\) found in milk volatiles, less acutely toxic to rodents by inhalation and intraperitoneally by a factor of 1.5–2.0 than the corresponding methylthiol; in contrast dimethyl disulfide \((\text{CH}_3\text{S} - \text{SCH}_3)\) is more toxic by a factor of 3 or 4 than methylthiol (23).

**Gas Mixtures**

Only recently has experimental attention been given to the physiological effects of mixtures of various chemical air pollutants, prompted by sifted evidence from acute episodes-indicating that the effects observed on health could not be explained by the action of single substances alone at the concentrations encountered. Mixtures of substances have been used since the beginning of clinical medicine, in an attempt to surpass the effects of exposure to a single component. Out of such trails emerged the findings of (1) antagonism (reduced effects), (2) synergism (enhanced effects), and (3) simple additive effects. Although Burgi (29) began a serious analysis of effects of mixtures in 1910, no investigation of the mechanism by which these combined effects are brought about has been found.

Not much research on the effects of mixtures of commonly found air pollutants has been done. The difficulties, both experimental and theoretical, of working with single substances are compounded manyfold when working with mixtures. Quantitative analysis of trace amounts of one substance in the presence of others often presents an experimental problem of great magnitude; while theoretically speaking, the question of the precise mechanism often remains unanswered due to the incomplete nature of the theory.
Dautreband (30) was among the first to recognize and study the interaction of solid aerosols on gases and vapors as an air pollution problem. His work, appearing in 1951, drew attention to the effect of particulate matter on eye irritation produced by the volatile irritants, sulfur dioxide, formaldehyde, and nitric acid vapor; and the importance of particle size in connection with air pollutant exposures.

La Belle et al. (31) show that a wide variety of aerosols of relatively large particle size can materially alter the pulmonary response of mice to respiratory irritant vapors, when administered together with the vapors at relatively high concentrations (50 - 2900 ppm). Thus, like Dautreband for the eye, La Belle et al. demonstrate an enhancement of effect by particulates for a different site of action, the lung. In addition, they show a reduced effect with certain mixtures while observing no effect with others.

In work demonstrating the combined effect of aerosols and irritant-gases, the physiological effects of a mixture of SO₂ and NaCl have been determined by Amdur (32) in unanesthetized guinea pigs. With concentrations of sulfur dioxide of 2 - 250 ppm and particulate sodium chloride of 12 ppm and of submicronic size (0.04 μ diameter), the irritant effect, measured by pulmonary resistance, was greater than for corresponding concentrations of sulfur dioxide used alone.

Goetz (33) has developed a physiochemical theory to explain the altered physiological effects of combinations of vapor and aerosol. Stated in its most elementary form, the physiochemical considerations that govern the final toxicological outcome are: (1) the degree of adsorption and absorption of the vapor on or in the particle, (2) the degree of chemical or catalytic interaction of vapor and particle, (3) the relative rate of desorption of the substance from the particulate onto the biological surface, and (4) the toxicity of the new combination, if such occurs.
This theory finds considerable support in the results of La Belle et al. (31) and Amdur (32), and may be used to explain not only synergism but antagonism between aerosol and vapor. For if adsorption of vapor on particulate occurs with a resulting new chemical compound of lesser toxicity, a lesser effect is predictable. On the other hand, if a vapor such as sulfur dioxide, which ordinarily does not penetrate into the lung because of upper respiratory absorption, is absorbed on a particle of respirable size, it will be carried to the lung in very highly localized concentrations. Then, if desorbed onto the mucous surfaces, a synergistic effect may be anticipated.

Undoubtedly, similar conditions operate to explain the enhanced effects of sulfuric acid mist when inhaled with sulfur dioxide, Amdur (34); 8 mg/m³ H₂SO₄ mist and 89 ppm sulfur dioxide inhaled as a mixture produced more marked effect on guinea pig growth, lung tissue changes, and respiration than was predicted from the simple added effects of each.

Hydrogen sulfide (H₂S) at a concentration of 400 ppm also materially increases the toxicity of 0.5% carbon monoxide. A 10-min. exposure of the mixture was fatal to mice, whereas the same exposure to each gas singly was not. Exposure to the mixture at one-half the concentration of each gas for longer periods was also fatal. This effect is possibly one of true synergism, although hydrogen sulfide at this concentration stimulates respiration and thus increases intake of carbon monoxide. In contrast to hydrogen sulfide, carbon monoxide is not rapidly fatal, and thus simple additive effects of each gas would not be expected.

In conclusion, it should be apparent that, although considerable insight has been gained into the effects of certain of the more prominent gases on animals, knowledge of the toxic potentialities of some of the more unusual gases is grossly incomplete and unsatisfactory. Similarly, only a beginning has been made in the
important area of toxicological interactions in which the presence of one air pollutant may, at one extreme, completely abolish the effects of another or, at the other extreme, enhance the effects out of all proportion to the toxicity of either alone.

Effects of Some Indigenously Produced Gases on Materials

The damage to non-living materials from polluted air has long been a significant source of economic loss in our society. Pertinent here is that in the agricultural industry, complete confinement of domestic animals has created highly corrosive environments which have prematurely destroyed building materials and equipment.

The more common mechanisms of deterioration in polluted atmospheres are:

1. **Direct Chemical Attack** - Air pollutants react irreversibly and directly with the material causing deterioration. Examples of this are the tarnishing of silver and the etching of a metallic surface by an acid mist.

2. **Indirect Chemical Attack** - The material absorbs pollutants but its deterioration results not from the absorbed pollutant, but from products of chemical conversion.

3. **Electrochemical Corrosion** - Much of the atmospheric deterioration of ferrous metals is by an electrochemical process. Numerous small electrochemical cells tend to form on ferrous metal surfaces exposed to the atmosphere whereby anodes and cathodes are set-up as the result of local chemical or physical differences on the metal surfaces. The presence or absence of moisture will largely determine the extent of corrosion by this means. Also air pollutants such as sulfur dioxide will destroy developed protective oxide films (35).
Larrabee (35) reports that when iron remains in dry air for an appreciable length of time it develops a protective film of oxygen, but that the subsequent presence of sulfur dioxide (SO$_2$) will destroy the protective oxygen layer.

Among the more important factors contributing to corrosion by air pollutants are moisture and temperature. Without moisture in the atmosphere there would be little if any corrosion even in the most severely polluted environments. Visible wetting of surfaces is not required for corrosion to take place. For several metals there seems to be a critical atmospheric humidity which, when exceeded, produces a sharp rise in the rate of corrosion. Sanyal and Bhadwor (36) report that for atmospheres containing sulfur dioxide (SO$_2$) aluminum has a critical humidity of 80% and mild steel shows two, 60 and 75%.

The most obvious influence of temperature is on the rate of the chemical reaction responsible for deterioration. Objects exposed during a radiation temperature inversion lose heat rapidly and usually cool to temperatures below that of the ambient air. If their surface temperature falls below the dew point condensed moisture forms. The resulting moist surface, in the presence of corrosive pollutants whose concentrations are increasing under the influence of the temperature inversion, provides an ideal environment for the promotion of damage.

Because corrosion of materials is in itself an extensive subject of study, discussions here are limited to a few of the compounds commonly encountered in agricultural atmospheres.
Carbon Dioxide

The principal undesirable effect of atmospheric carbon dioxide is deterioration of masonry work (37). In the presence of moisture, carbon dioxide produces carbonic acid, which attacks calcium carbonate, converting it to the water-soluble bicarbonate which is then leached away. Carbon dioxide is also responsible in part for the atmospheric corrosion of magnesium.

Sulfur Oxides

Moisture must be present for sulfur oxides to attack iron and steel and there appears to be a critical humidity above which the corrosiveness of sulfur dioxide is accelerated. Figure 1 summarizes the recent work of Sereda (38) and relates corrosion of steel to outdoor concentrations of sulfur dioxide.

Figure 1. Graph shows how the rate of corrosion of steel based on a day of wetness is affected by the temperature and sulfur dioxide pollution rate (38)
Work by Binger et al. (39) shows aluminum to be fairly resistant to attack by sulfur oxide concentrations normally found in polluted atmospheres.

**Hydrogen Sulfide**

Hydrogen sulfide can be oxidized in the atmosphere to SO$_2$ and SO$_3$ especially under conditions of high humidity. However, there are several forms of deterioration which are related directly to H$_2$S gas.

Paints that contain lead compounds, for example, are rapidly darkened in the presence of even low concentrations of H$_2$S by the formation of black lead sulfide.

**Solid Particulates**

Particulate matter appears to be an important factor in the corrosion of metals, particularly in the presence of an acidic forming gas. In work carried on at the Chemical Research Laboratory in Teddington, England (40) it was shown that the rusting of iron in a moist atmosphere containing SO$_2$ is greatly accelerated by the presence of particulate matter. In one experiment a sample was exposed to a moist atmosphere containing traces of sulfur dioxide (SO$_2$). Another sample was exposed to the same atmosphere, but protected from particulate matter by means of a muslin cage which permitted only the gaseous constituents to contact the sample. The rusting of the protected sample was negligible while the unprotected sample rusted severely.

The same effect was confirmed by Preston and Sanyal (41). Various metallic surfaces (coated and uncoated) were "inoculated" with fine powders using such
materials as sodium chloride, ammonium sulfate, ammonium chloride, sodium nitrate, and flue dust. The samples were then exposed to atmospheres held at various humidities and the resulting corrosion was measured. With all but one of the inoculates corrosion increased with humidity, the exception being ammonium chloride.

Barton (42) carried out experiments to determine the corrosive effects of a variety of artificial dusts on metals. He concluded that the quantity of $H_2O$-soluble in the dusts, the pH of the resulting solution, and the concentration of chloride and sulfate ions were important factors in this type of corrosion. He stated that, contrary to previously expressed opinions, corrosive action was not affected by dusts with high absorptive capacities for water and sulfur dioxide.

In conclusion, fragmentary data on the effects of air pollutants and aerosols on the performance of animal physiology and material durability is available. Physiological changes that isolated pollutants will induce in test animals under given concentrations of various gases have been observed. Evidence has also been obtained on the corrosion encountered with some specific combinations of air pollutants and materials.

Very little information is available on the effects of mixtures of various air pollutants, either combined or uncombined with particulate matter, on animal and material performance. Although little research has been done in this area, the importance of the problem is well recognized along with the associated findings of synergism and antagonism.
The evidence presented thus gives only an insight into the nature of air pollutants and the importance of studying their detection, their source of origin, and the effects they have on both animals and buildings.
The origin and types of many gases that might occur in the atmosphere of a livestock building have been identified and characterized within the disciplines of organic and biochemistry. Their normal characterization, through preparation and yield of organic compounds, however, is not as important to this study as is their reaction in a dilute solution when serving as a source of energy for living organisms. Similarly, classes of, rather than specific individual compounds are of most interest.

Organic Decay and Decomposition

Waste organic substances of all types, including grasses, leaves, manure, human and industrial waste, are utilized as a source of energy by a succession of living microbiological organisms. A series of biochemical reactions are set in motion and eventually the waste materials are decomposed and returned to nature. This decomposition of organic matter is brought about by a highly complex bacterial metabolism.

The changes through which organic constituents of animal wastes are metabolised by bacterial life is termed biolysis. The bacteria break down the complex organic substances, such as carbohydrates and proteins, into simpler organic acids and then ferment these acids to ultimately form methane and carbon dioxide.

The first step in the breakdown of animal wastes is marked by the rapid disappearance of the available oxygen. Urea, ammonia, and other products of digestive putrefactive decomposition are partially oxidized, rapidly consuming the available oxygen and causing the wastes to become anaerobic. The second state is putrefaction in which the action is under anaerobic conditions. Proteins are broken down to form urea, ammonia, the foul-smelling mercaptans, hydrogen sulfide,
aliphatic and aromatic acids, amines, and amides; fats are broken down into their original fatty acids, water, carbon dioxide, hydrogen, methane, and other substances; carbohydrates are affected somewhat similarly to the hydrocarbons, turning to alcohols, aldehydes and then acids, together with carbon dioxide, hydrogen, methane, and other compounds. The third state is the oxidation or nitrification of the products of decomposition resulting from the putrefactive state into nitrates and nitrites, which are stable and usable forms of plant food.

Because the anaerobic second stage of biolysis, putrefaction, gives off foul-smelling odors, the gases produced are of special interest. The gases that have been detected and studied thus far are methane, carbon dioxide, ammonia, hydrogen sulfide, and sulfur dioxide. Day et al. (4) have detected and measured these gases in relationship to management, ventilation, and building design of hog houses. McAllister (6), in reporting on research of dung gases in buildings, points out numerous cases of gas poisoning, reduced productivity, and even deaths that have been attributed to the above mentioned gases.

It appears, however, that other gases are present in the composition of a livestock atmosphere which create obnoxious odors and potential health hazards for animals and their managers. To point out what gases these might be, it becomes essential to look briefly into the biochemistry of carbohydrates, proteins and fats, and to trace the metabolic pathways to see what chemical changes are brought about by the biolysis of animal wastes.

The main pathways along with the major intermediate products of animal wastes are schematically (43),
Other intermediate products that may result from the biological degradation of the amino acids, fatty acids, and alcohols, are mercaptans, amines, amides, carbonyls, and esters.

Carbohydrates

The term carbohydrate applies to all compounds of carbon, hydrogen, and oxygen in which the hydrogen and oxygen are in the ratio of 2:1. Carbohydrates may be grouped into three general classifications depending upon the complexity of their structure: (1) simple sugars, or monosaccharides; (2) complex sugars, or disaccharides; and (3) polysaccharides.

Among the monosaccharides, glucose is of particular interest in waste treatment. It contains a carbonyl group in the form of an aldehyde and is usually the sole product when di- or polysaccharides are hydrolyzed. This sugar is readily oxidized by aerobic bacteria and is also rapidly fermented under anaerobic conditions with acid formation resulting in both cases.
Two polysaccharides of interest in the processing of animal wastes are starch and cellulose. Starch has the general formula \( (C_6H_{10}O_5)_n \) and occurs in a wide variety of products grown for food purposes, such as; corn, wheat, potatoes, etc. Cellulose has the same general formula as starch but differs in its structure. Cellulose forms the structural fiber of many plants and thus is contained in most animal waste products. In both cases, biological degradation presumably involves hydrolysis to glucose as the first step. The molecules of glucose are further reduced by bacterial action to alcohols, aldehydes and ketones, organic acids, and finally to carbon dioxide, methane, and water.
Proteins

Proteins are complex structures containing carbon, hydrogen, oxygen, and nitrogen with a few containing phosphorous and sulfur. Like polysaccharides, which may be considered to be made of glucose units, proteins are formed by the union of -amino acids. Amino acids, the building blocks of protein, all have an amino group (\(-\text{NH}_2\)) attached to the alpha carbon atom as well as an acid radical (\(-\text{COOH}\)) and, thus derive their name of amino acids.

The biological degradation of proteinaceous matter takes place through hydrolysis, progressing in steps until the protein is broken down into amino acids. It is essential for hydrolysis to yield amino acids as an end product so that passage through the cell walls is possible.

Hydrolysis of protein to amino acids proceeds according to the pathway

\[
\begin{align*}
\text{PROTEIN} & \rightarrow \text{PROTEOSES} \rightarrow \text{PEPTONES} \rightarrow \text{POLYPEPTIDES} \rightarrow \text{DIPEPTIDES} \rightarrow \text{AMINO ACIDS}
\end{align*}
\]

Within the cell, deaminization of the amino acids occur resulting in the production of free fatty and other organic acids. The nature of deaminization varies according to whether aerobic or anaerobic conditions exist.

Under aerobic conditions deaminization of amino acids by bacteria produces either saturated acids with one less carbon atom or hydroxy acids with the same carbon atom.
Bacterial deamination under anaerobic conditions may proceed with or without reduction to form the corresponding saturated or unsaturated acid.

These acids formed under aerobic or anaerobic conditions are further oxidized as free or fatty acids producing acetic acid.
Fats

Fats, like carbohydrates, are made up of carbon, hydrogen, and oxygen and are esters of the trihydroxy alcohol, glycerol. Bacteria use fats as a source of food, hydrolyzing them to the corresponding fatty acids and alcohols. These free fatty acids and those produced in the deamination of amino acids undergo further breakdown according to Knoop's beta-oxidation theory in which acetic acid is cleaved from the original acid. Acetic acid is oxidized to carbon dioxide and methane.

Metabolic Pathways

Thus far, the biochemistry concerning the initial steps in the degradation of waste matter has been presented. The presentation was oversimplified, its purpose only to point out the intermediate breakdown products of the three main organic substances of carbohydrates, proteins, and fats. These intermediate products then enter a common terminal pathway enroute to their final stages of decomposition. Some of the seemingly more pertinent metabolic reactions follow.

Saturated hydrocarbons and glucose molecules break down to form an alcohol and then enter into a common pathway. The saturated hydrocarbon converts to an unsaturated hydrocarbon which reacts with water to form an alcohol. Glucose, through the process of glycolysis, also breaks down to form an alcohol. The alcohols are oxidized to the aldehyde and then to an acid. The basic metabolic pathway is
If the starting material is ethane, acetic acid would result, which is the key intermediate in all biological metabolisms. If the starting material contains three carbons or more, the acid formed must be further metabolized.

The currently accepted scheme for the metabolism of higher organic acids resulting from (1) the degradation of hydrocarbons and carbohydrates; (2) the deamination of amino acids; and (3) the metabolism of fatty acids is the beta-oxidation theory which cleaves an acetic acid molecule from the original organic acid.
The enzyme system involved in this cleavage contains Co-enzyme A (CoA). CoA reacts with the acid to form a CoA-acid complex. Desaturation occurs between the alpha and beta carbon. Water is added to the molecule to form a \(-\)hydroxy acid. Oxidation forms a keto acid which is split by CoA to form acetyl-CoA and an acid-CoA complex, which now has two carbon atoms less. The acetyl-CoA can be oxidized to carbon dioxide and water while the other component, if larger than a two carbon complex, undergoes further degradation. The pathway for the beta-oxidation process is

\[
\begin{align*}
\text{Acid} + \text{HSCoA} & \rightarrow \text{Acyl-CoA} + \text{H}_{2}\text{O} \\
\text{Acetyl-CoA} + \text{HSCoA} & \rightarrow \text{Keto-CoA} + \text{\(-\)Hydroxy Acid} \\
\end{align*}
\]

Besides the intermediate and end products produced by the metabolic pathways, there are other intermediate organic reactions taking place which result in still other compounds. Among these reactions are those yielding esters, amides, amines, and sulfur derivatives.
Esters

Esters are compounds formed by the reaction of acids and alcohols. The reaction may be represented as:

$$R\text{-COOH} + R\text{,OH} \rightarrow H_2O + R\text{—C—O—R}$$

Acid Alcohol Ester

Esters are hydrolyzed by micro-organisms to yield the corresponding acid and alcohol which is then reduced according to the metabolic pathways to carbon dioxide and water.

Amides

The amides are derived from organic acids and ammonia. Urea is an amide of considerable importance because it is a normal constituent of urine. Urea is hydrolyzed to carbon dioxide and ammonia according to the following reaction:

$$\begin{align*}
\text{NH}_2 \\
\text{C = O} & + \text{H}_2\text{O} \rightarrow \text{CO}_2 & \text{2NH}_3 & \\
\text{NH}_2 & & \\
\text{UREA} & & \\
\end{align*}$$

Amines

The amines are alkyl derivatives of ammonia (R-NH$_2$). They are found in nature particularly in the fish and beet industry. Therefore, if animals are fed fishmeal, amines may be present in their waste matter. Amines may also be present due to the decarboxylation of amino acids. The mechanism of this enzymic reaction, catalyzed by pyridoxal phosphate, is
The net result is the production of carbon dioxide and a primary amine, whose formula can easily be derived from the amino acid which was decarboxylated.

**Sulfur Compounds**  
Sulfur molecules are present in the amino acids of cysteine and methionine. These two compounds are degraded chiefly through one of two reaction sequences: either to pyruvate plus hydrogen sulfide and ammonia or through oxidation to cysteic acid and subsequent decarboxylation and deamination resulting in a mercaptan. The pathway of this degradation is

\[
\begin{align*}
\text{NH}_3 + & \text{CH}_3 + \text{CH}_2 - \text{SH} \\
\text{H}_2\text{S} + & \text{COOH} + \text{COOH} + \text{CH}_2 - \text{SH} \\
\rightarrow \text{DECARBOXYLATION} & \rightarrow \text{DEAMINATION} \\
\end{align*}
\]

Upon further degradation these compounds enter the waste matter in the form of sulfates. In the absence of dissolved oxygen and nitrates, sulfates serve as a source of oxygen for biochemical oxidations produced by anaerobic bacteria. Under anaerobic conditions, the sulfate ion is reduced to sulfide ions which establishes an
equilibrium with the hydrogen ions to form sulfides.

\[
\begin{align*}
\text{SO}_4^{2-} + \text{ORGANIC MATTER} & \xrightarrow{\text{ANAEROBIC BACTERIA}} \text{S}^2 + \text{H}_2\text{O} + \text{CO}_2 \\
\text{S}^2 + 2\text{H}^+ & \rightarrow \text{H}_2\text{S}^\uparrow
\end{align*}
\]

In summary, the known biochemistry and metabolic pathways of bacterial reduction of livestock wastes would indicate that several gases might be found in the atmosphere of a confinement livestock production building. Of these, the more important to this study are the low molecular weight organic compounds, namely the straight-chain hydrocarbons, alcohols, aldehydes, ketones, esters, amines, mercaptans, etc.; and a few inorganics, such as carbon dioxide, ammonia, hydrogen sulfide, and sulfur dioxide.

Each compound, upon entering the atmosphere, establishes its own specific equilibrium condition between the liquid and gaseous phase. The concentration of each gas will vary according to its vapor pressure at a given temperature. The number and types of gases present in the building at any given time will depend upon the size and age of the animals, the strength and age of the wastes, and the waste disposal system employed in the building.

In reviewing the general metabolic and organic reactions in the preceding sections, only a few key reactions are found. Consequently, though the pathways as reported are a gross oversimplification of the complex series of reactions, with them one can follow the degradation process and predict the products present in the waste matter and what gases might be expected to exist in a confined livestock building.
A first step in the systematic identification of an organic substance is to gather some general behavioral information on the unknown and then to seek, by qualitative tests, the compounds present. Generally there exists a variety of possible procedures and tests seeking this general information, many of which may give valuable clues to the nature of the unknown.

**Solubility**

The solubility of organic compounds in selected solvents was chosen as the first step for identification purposes in this study. On the basis of solubility behavior of an unknown, decisions can be made as to a logical sequence for identifying the functional groups present.

The outline for classifying the unknown gaseous components according to the solubility behavior as described by Cheronis et al. (44) is:

```
THE UNKNOWN GAS
  | SOLUBLE
  | WATER
  | SOLUBLE
  | 2 N HCl
  | INSOLUBLE
  | SOLUBLE
  | 2.5 N NaOH
  | INSOLUBLE
  | CONC. H_2SO_4
  | INSOLUBLE
  | 1 N NaHCO_3
  | SOLUBLE
  | INSOLUBLE
  | DIVISION A1
  | SOLUBLE
  | INSOLUBLE
  | DIVISION A2
  | SOLUBLE
  | INSOLUBLE
  | DIVISION N
```

The absorption train employed in the solubility classification technique is
When liquid manure is placed in a 5 liter round-bottom flask and a stream of nitrogen bubbled through the manure, organic vapors are forced through the absorption traps. Each absorption trap contains 25 ml of a reagent. After 24 hours of operation qualitative tests are conducted on each trap according to its solubility classification. In the system shown, eight functional groups are considered: amines, amides, acids, alcohols, esters, carbonyls, sulfides and mercaptans.

These tests indicate, with considerable assurance, the presence of certain chemical classes. Systematic methods may now be used to concentrate and collect these groups for additional analyses.

Gas Chromatography

Gas chromatography is a currently useful way to follow up the findings of solubility tests because it affords a way to separate even minute components in an unknown mixture, each component characterized by an almost unique retention time under a defined set of operating conditions. Several techniques for analysis by gas chromatography seem specifically applicable to this study and a brief description of each follows.
Identification by Occurrence of Peaks

For the investigator who must deal with entirely unknown mixtures of compounds, the use of retention data alone for the identification of components separated by gas chromatography is a serious limitation. In most cases, the mixtures encountered contain a number of heterofunctional and isomeric components, and some of the components of such mixtures have the same, or nearly the same, retention times, even through different column materials. Although the analysis ultimately might be accomplished by finding suitable liquid phases to effect resolution and identification, the large number of liquid phases to be studied make this approach time-consuming and unrewarding. Even though the problem has been solved in some cases by collection of the eluted peaks followed by infrared spectrophotometry (45, 46) or mass spectrometry (47, 48) for subsequent identification, the additional instrumentation is very expensive and the collection procedure is cumbersome and not entirely reliable.

The failure of gas chromatography to provide complete qualitative analysis then is due primarily to the inability of the gas chromatograph to determine or distinguish organic functionality (49). Therefore, if the functionality can be independently established, the identity of a particular compound can now be made from its retention volume. Various methods have been undertaken to develop the systematic use of functional group classification reagents to provide direct, rapid, and inexpensive methods of qualitative gas chromatographic analysis.

Among these techniques is work done by Walsh and Merritt (50) to identify the eluted peak by a reaction test with a set of functional group classification reagents. Because the eluted gas in chromatographs using flame detectors is destroyed by being burned, the addition of a stream-splitter is required which by-passes a portion of the eluted sample to a set of classification reagents.
Another method by Bassette and Whitnah (51) removes certain classes of compounds selectively from the original mixture and causes a corresponding disappearance of peaks from the chromatograms of the head space gas above the mixtures.

Hoff and Feit (52) devised still another technique in which prechromatographic reactions between vapor mixtures and selective reagents takes place in a hypodermic syringe. This method either eliminates functional groups of compounds or converts them to other families which can be easily detected on the chromatogram.

A combination of the afore-mentioned methods may be employed to distinguish organic functionality in a gas sample containing a number of heterofunctional components. Once the functional group of an eluted peak is established, it can then be further identified by means of its retention time or by a semi-log plot of retention time versus carbon numbers.

Identification by Elimination of Peaks

This technique, described by Hoff and Feit (52) as the syringe reaction technique, is successful in eliminating carbonyls, amines, and acidic compounds from gaseous samples. The principle involves bringing the dilute vapors of organic compounds into contact with selective chemical reagents inside a hypodermic syringe before injection into the chromatograph. This technique is especially useful in identifying the carbonyl compounds in an organic gas sample collected by the salting concentration technique.

In the salting concentration technique, carbonyls are eliminated by treating the gas sample with a hydroxylamine reagent, prepared by adding 4 gms. of NH$_2$OH . HCL to 50 ml. of water. To the inner wall of a 5 ml. syringe, 25 µl. of the reagent is applied and the plunger inserted in a rotary manner to insure an even wetting of the syringe wall. The gas sample is drawn into the syringe and exposed for three minutes to the reagent before injection into the chromatograph. The eliminated peaks on the
treated chromatogram, compared with the untreated chromatogram, identify the carbonyls.

Sodium bicarbonate and hydrochloric acid reagents also may be used for the detection of organic acids and amines, respectively.

**Identification by Conversion to Other Compounds**

This technique also is described among the syringe reactions by Hoff and Feit (52) and is used to identify alcohols by converting them to their corresponding nitrites. Again, these samples are collected by the previously described salting concentration technique.

In this case, the syringe is treated with 25 μl of a sodium nitrite reagent and exposed for three minutes before injection. The sodium nitrite reagent consists of a mixture of equal parts of freshly prepared 2.5 g of NaNO2 in 50 ml of H2O and 1 N H2SO4.

The nitrite peaks are easily identified as sharply eluted peaks of short retention times.

**Identification by Selective Absorption**

Group functionality of the unknown gases can also be established by the use of absorption traps. These traps contain reagents that will selectively absorb or form derivatives of a specific chemical group. The individual compounds of the group can then be further identified as derivatives of the reagent or as pure compounds if they are regenerated back to their initial state.

This technique is employed in the concentration and identification of alcohols. Volatile gases from a manure sample are bubbled through the absorption train and the alcohols collected as their nitrites in the sodium nitrite reagent. This solution is
injected directly into the chromatogram and the alcohols identified as their nitrite derivatives.

It should be noted that this technique has a two-fold purpose in that it not only classifies the group but also can be used to concentrate the gases. As a result, two methods that have been used to concentrate specific groups also serve to aid in their identification. These methods, previously discussed in more detail under the section titled Concentration by Selective Absorption, are used to concentrate and identify alcohols and carbonyl compounds.

**Determination of Compounds Within a Homologous Series**

The gas chromatograph has the ability to separate complex mixtures into individual components. The identification of these compounds often times becomes a very difficult task. If, however, the peak can be classified as a member of a given chemical group, the final identification is considerably simplified. Two methods that work very well for identifying the components in a homologous series are retention time data and log plotting.

With retention time data, the volume of carrier gas required to elute a compound from a gas chromatograph column is called the retention volume. Because the flow rate is linear with time it can also be referred to as the retention time. The retention time is characteristic of the sample and the liquid phase, and therefore can be used to identify the sample. Identification is based on a comparison of the retention time of the unknown component with that obtained from a known compound analyzed with the same column under identical conditions.

With log plotting, if a sample containing several members of a homologous series is injected into a gas chromatograph, the log of their retention times is proportional to some increasing property of the homologous series. Therefore, identification
of members of a homologous series can be obtained by plotting log of the retention
time versus the number of carbon atoms.

This method of identification is useful in that only two or three compounds are
needed to establish the slope of the line and thus can be used to identify other
members of the series.

**Miscellaneous Techniques**

There are, in addition to the tests and techniques mentioned, other selective
methods for the identification of certain compounds. A few of these methods that are
especially helpful will be mentioned.

A method to absorb hydrogen sulfide and methyl mercaptan was developed by
Koren and Gierlinger (53). The reagent is prepared from bismuth nitrate and capable
of selectively precipitating out these two compounds. The procedure for preparing
the reagent is presented in Appendix C.

Another method used to selectively absorb hydrogen sulfide and methyl mercaptan
was developed by Marbach and Doty (54). In this case, the odorous vapors were
carried with a stream of nitrogen into a trapping tube containing cadmium hydroxide
and sodium hydroxide. Methylene blue is then developed by adding a mixture of the
acid amine solution and Reissner’s solution (see Appendix B) to the trapping tube and
vigorously shaking. The two gases can be identified by measuring their absorption band
on a photoelectric colorimeter; 665 m\(\mu\) for hydrogen sulfide and 490 m\(\mu\) for the methyl
mercaptan. Quantitative analysis of the gases is easily performed if standards are
developed.

Disulfides can be collected by bubbling the odorous vapors through a reagent trap
containing mercuric acetate (4% W/V). A white precipitate is formed by the addition
of sodium chloride which can be used to regenerate the gases for chromatographic
analysis (55).
EXPERIMENTAL

General

The experimental portion of this research was conducted in the AISI-ISU Swine Atmosphere Research Building, a laboratory cooperatively supported by American Iron and Steel Institute and Iowa State University. The building contained two animal chambers, an air-mixing chamber, a mechanical and feed room, and a laboratory (Fig. 2). In addition to the work described in this study, the facilities were equipped to conduct exposure studies on selected materials for floor, pen partitions and room surfacing construction (Fig. 3). Additional details of the facilities are presented in Appendix A.

Each animal chamber had capacity for 24 animals and was equipped to provide substantial control over the environmental conditions. Some variations in management practices also were possible to meet the needs of the experiment. The building was occupied by animals throughout the year except for short periods of time between experimental groups. At this time the entire facility was cleaned.

Entering hogs averaged about sixty pounds each. They were divided into equal groups of eight pigs and placed in the three pens in each chamber. The hogs received a self-fed 14% protein growing-finishing ration and were kept in the building until they reached market weight. The main purpose of the hogs was to create a typical swine atmosphere under controlled conditions that could be analyzed for its specific components.

Each pen was four feet wide by sixteen feet long, had a floor slope of one-half inch per foot and contained a four feet square section of slotted floor at its lower end. A deep narrow gutter beneath the slotted floor area, equipped with an overflow pipe, served as a manure storage pit. The pits were drained and cleaned every two to three
Figure 2. The research facility

Top: AISI-ISU Swine Atmosphere Research Lab.
Center: Analytical Lab.
Bottom: Animal Chamber.
Figure 3. Exposure tests

Top: Wall samples
Center: Pen partitions
Bottom: Slotted floors
weeks depending upon the size of animals. Chamber management approached that of a commercial hog enterprise.

The temperature in each chamber was maintained at 70 degrees Fahrenheit or above to promote a high odor production. A pressurized ventilation system provided a supply of fresh air at all times to each chamber.

The laboratory space was maintained at a relatively constant temperature throughout the year to minimize variability in testing procedures. Two gas chromatographs were used in the study. One chromatograph was equipped with a hydrogen-flame ionization detector while the other system contained a thermal-conductivity detector head.

The Gas Analyzing Systems

Hydrogen-Flame System

The primary component of the hydrogen-flame system was an Aerograph Model 660 gas chromatograph equipped with a single channel electrometer, a hydrogen-flame ionization detector and a strip-chart recorder. The chromatograph has separate, independently heated and controlled ovens for the detector and column, and a separate, independently heated and controlled injection port. The temperature for all three components can be read from a pyrometer on the chromatograph.

The gas flow system for the Aerograph 660 is shown schematically in Figure 4. A carrier gas supplied by a high pressure supply tank flows through a two-stage regulator, a flowmeter, a flow controller, and a 6-way gas sampling valve to the heated injector. Samples can be introduced into the system either through the 6-way valve or by means of a syringe and needle through a rubber septum on the heated injector port.
Hydrogen gas to operate the detector head also flows through a two-stage regulator, a flowmeter, and an inline flow restrictor to the detector head.

After a sample has been injected, it is transferred by the carrier gas into the column where separation of the unknown gas sample takes place. The separated components then flow through the detector producing an electrical output which is amplified and recorded.

Nitrogen was used as the carrier gas in this system and was metered along with the hydrogen flow by two calibrated Brooks Sho-Rate "150" Rotameter-Kits. These rotameters provided a continuous check of the flow rates to the gas chromatograph.

The Hydrogen-Flame Ionization Detector

The hydrogen-flame ionization detector (FIID), shown in Figure 5 and used in this study, for the detection of organic gases, is based on the principle of ionization of the solute and measurement of the current generated. This type of detector characteristically possesses a large linear range and is applicable with high
sensitivities to a wide range of solutes. It is relatively insensitive to flow and temperature changes, and is very rugged and reliable, requiring only minimum maintenance. It is also insensitive to fixed gases, namely, H₂S, CO₂, SO₂, CO, and H₂O.

Figure 5. Schematic cross section of the hydrogen-flame ionization detector

Figure 6 shows the schematic circuit of the flame ionization detector. According to Gill, et al. (56),

"Hydrogen is burned in air to obtain a plasma which has a sufficient thermal energy to ionize the organic matter passing through it. The ions generated are collected at a polarized electrode closing the electrical loop shown. The resulting ion current is monitored by measuring the voltage drop across the resistor."

"The sensitivity of the flame ionization detector is roughly proportional to the carbon content of the solute, i.e., with hydrocarbons the normalization of peak areas gives percentages very close to the weight composition. Sensitivity varies with other compounds and requires calibration for high accuracy."
Thermoconductivity System

The primary component in the second gas analyzing system was an Aerograph Model 90-P3 gas chromatograph equipped with a thermoconductivity detector. The 90-P3 has individual temperature control for the injector vaporizer, column oven, detector oven, and collector exits. The temperature of all of these components can be read out on a pyrometer on the chromatograph.

The gas flow system for the 90-P3 is shown schematically in Figure 7.

Figure 6. Schematic circuit of the flame ionization detector

Figure 7. Gas flow system for the 90-P3
A carrier gas flows from a high pressure supply tank through a two-stage regulator, a flowmeter, a flow controller, and an Aerograph 6-way sampling valve to the heated injector. This system, like the hydrogen-flame system allows either automatic injection by means of the 6-way gas sampling valve or manual injection by means of a syringe and needle.

Helium was used as the carrier gas in this system and was metered by means of a Brooks Sho-Rate "150" Rotometer.

The Thermoconductivity Detector

The differential thermoconductivity detector responds to all compounds except the carrier gas itself. It is useful for analyzing mixtures of inorganic and organic compounds.

The detector consists of four \( Vv'X \) (tungsten-rhenium) filaments each of which compose the elements of a bridge circuit for maximum sensitivity. The Wheatstone Bridge along with the power supply is solid state and includes a bridge balance, attenuator controls, milli-ammeter to indicate the filament current and coarse and fine zero adjust to set the electrical zero of the recorder.

A major advantage of the thermoconductivity detector is that a sample is not destroyed by the detector thus permitting collection of the separated sample for other identification procedures.

Making the Equipment Operational

This section describes the procedure required to place the systems into operation before any gas analyses could be conducted. This procedure is applicable to both the Model 660 and the 90-P3 chromatograph.
The day before a test was to be conducted, the chromatograph injector, column, and detector ovens were set to the selected operating temperatures and brought to equilibrium. (The temperatures in the various ovens were maintained by means of the equal heat-gain, heat-loss principle with specific settings on the variable transformer. Calibration curves were prepared to determine the oven temperatures for various transformer settings under a constant ambient temperature.) The electrometer and recorder were also turned on at this time and in the case of the Model 660 Chromatograph, the hydrogen flame was ignited. In all of the tests, the detector and injector temperatures were maintained above the column temperatures to prevent condensation of the sample in either the injector port or the detector head.

After the chromatograph ovens reached equilibrium the carrier gas and hydrogen gas flow rates were set according to rotometers, previously calibrated for each individual gas by means of a bubble flow meter. In the case of the Model 660, the carrier gas flow rate was adjusted to 30 ml/min. at 40 psig and the hydrogen flow rate was also set at 30 ml/min. but with a pressure setting of 10 psig. The carrier gas flow rates for the thermoconductivity detector varied with different tests, the setting reported on each chromatogram of the fixed gases.

Once the equipment became operational, it was maintained in this condition throughout the conduct of the test. Periodic checks were made to correct for any gas leaks or variations that might have developed during the testing period.

Each day, following a number of tests, the column was baked out to clear it of any residual gases in the column. To do this required disconnecting the exit end of the column from the detector head, and with the carrier gas still flowing, increasing the column oven temperature, and sustaining these conditions for at
least four hours. The temperature of the column oven for the bake out procedure
must be regulated to be above the operating temperature of the column under test
conditions but below the maximum temperature limit of the stationary phase.

When an unstable baseline appeared on the recorder, the column was steam
cleaned. Injection of 2 to 3 micro-liters of distilled water into the column created
a mass of moving steam through the column and detector head carrying along with it
water-soluble residual chemicals that might have been retained on the column
packing. However, this cleaning method is applicable only when the stationary
phase in the column is not water-soluble.

The Column

One of the keys to successful analysis of a mixture of various compounds by
gas chromatography is the selection of a proper column. In gas-liquid chromato-
graphy, the column consists of an inert solid material supporting a thin film of a
nonvolatile liquid in a tube. The tube, made of glass or metal, is coiled to fit
into a chromatograph oven. The choice of solid support, type and amount of liquid
phase, method of packing, length and temperature of the column all are important
factors in obtaining the desired separation of the chemical mixture to be analyzed.

Column Selection

There seems to be no fool-proof method for selecting the proper column for the
separation of a mixture of various compounds. Many complex separations are reported
in the various literature, using numerous chemicals and conditions. These literature
references, along with experience and/or trial and error methods, perhaps provide the
best guide to the selection of the proper liquid phase and column conditions. As a
general rule, the liquid phase should be similar chemically to the components of the mixture for an efficient separation.

The solid support The solid supports used were Chromosorb P, Chromosorb W, and Chromosorb W (AW-DMCS) (Acid Washed - Treated with Dimethyl dichlorisilane). The Chromosorb P has the advantage of a large surface area but contains active sights which result in tailing of the peaks. To reduce tailing, a high percentage of liquid phase is used, which in turn decreases the efficiency of separation of the column.

Chromosorb W, although possessing a smaller surface area, has fewer sights and therefore has a greater ability to resolve polar compounds with a minimum of tailing. These characteristics of Chromosorb W permit lower loaded liquid phases (2-10%) and thus more rapid analysis.

Chromosorb W (AW-DMCS) decreases tailing of polar compounds still further. However, water vapor in gas samples or aqueous solutions readily ionize the salt and cause a complete breakdown of the column.

The liquid-phase The liquid phases employed in this study include Carbowax 20M and UCON 50 HB 2000 Polar. Carbowax 20M is a trademark of Union Carbide and is a polyethylene glycol polymer. It is especially useful for separating polar and semi-polar compounds. The loading rates used were 30% concentrations on Chromosorb P and 5 to 10% concentrations on Chromosorb W.

UCON 50 HB 2000 Polar is also a Union Carbide product designating polymers of polypropylene glycol. The loading rates for UCON were 5-10% on Chromosorb W.

Another column material used in this study was Poropak, a new polymer column packing by Aerograph that serves both as the liquid phase and solid support. One of the remarkable properties of this column is the rapid elution of water and other highly polar molecules with little or no tailing. The elimination of the conventional liquid
phase also reduces bleeding of the column. "Bleeding" refers to the loss of the column liquid phase and its transport to the detector head where it creates a voltage disturbance, termed noise, on the strip-chart record.

Column Preparation

The columns used were coiled pieces of stainless steel tubing, one-eighth or one-fourth inch in diameter and six feet long. Each column was provided with two Swaglok nuts at each end for connection to the oven of the chromatograph.

The column packing was prepared by placing the weighed solid support in an evaporating dish and then adding the calculated amount of the liquid phase dissolved in a suitable volatile solvent. The amount of solvent used was just enough to create a slurry. The solvent was evaporated and during drying, the mixture gently agitated by shaking the dish. The resultant impregnated support was then ready for packing.

In packing a column, a straight piece of tubing of the desired length and diameter was plugged loosely at one end with a piece of glass wool. A funnel was attached to the open end and filled with the coated support. The column was vibrated by a hand vibrator to facilitate the movement of the impregnated granules into the tubing. After the tubing was completely packed, a piece of glass wool was inserted into the open end. The packed column was then coiled around a pipe of suitable diameter and shaped to fit the chromatographic oven.

Conditioning of the Column

All columns, before being used for the separation of gases, were conditioned to eliminate bleeding. Conditioning the column consisted of disconnecting the exit end of the column from the detector and baking the column for 2-3 days in the column oven, as described previously, while a small amount of carrier gas flows through the column.
Preparation and Collection of Samples for Chromatographic Analysis

Many of the gases causing odors in a swine atmosphere may be present in such minute quantities that their mechanical detection is quite difficult. Even though present in concentrations as low as 0.1 ppm, some may still exert a pungent odor and cause physiological disorders. It is believed that the major components of odors in a confined livestock building result from fresh or partly decomposed manure. Initially samples were collected for analysis by drawing a 1 ml sample of the atmosphere from the space above the manure pits in the animal chambers. The sample was collected with a syringe and needle and injected directly into the chromatograph. The gases of interest, however, were found to be below the concentration levels detectable by the available chromatographic equipment, and as a result, methods had to be incorporated to concentrate the gases. Because this study was concerned first with a qualitative analysis of the atmosphere, use of concentration techniques would not affect the outcome of the experiment while it would facilitate the detection and identification of the various gases and greatly improve the reliability of the data. The concentration techniques used included methods of salting, condensation, and selective absorption.

Concentration by Salting

The analysis of gas above dilute aqueous solutions of organic compounds by gas chromatography has been reported by a number of workers (57, 58, 59). A technique that makes possible the direct chromatographic analysis of volatiles from biological fluids at concentrations of 1 ppm or less has been developed and reported by Bassette and co-workers (60). Although the method was developed for the detection of volatile components in biological fluids such as milk, blood, and urine, it was quite
easily adaptable for this study as the enrichment procedure for the organic gases.

The procedure involves the salting out of the dissolved gases from an aqueous solution by the addition of anhydrous sodium sulfate. Steps to prepare gas samples by this method are as follows: 1.2 grams of anhydrous sodium sulfate are placed in a 5 ml. serum vial and 2 ml. of a filtered liquid manure sample added. The vial is sealed with a serum cap and the solution vigorously shaken for 5 minutes. The vial is then immersed in a 60° C. water bath to a depth slightly above the level of liquid in the vial for 3 minutes. A needle from a gas-tight syringe is inserted through the rubber cap and the syringe evacuated and refilled five times to insure a good transfer of the head space gas into the syringe. The syringe is then removed from the vial, the volume adjusted to 1 ml., and the sample injected into the chromatograph.

Concentration by Fractional Condensation

Another method used to concentrate the volatile gases obtained above the holding pit was to pass the vapors through a cold trap and collect the condensed gases. Day et al. (61) used this technique successfully in identifying the volatile components of skimmilk.

The apparatus for collecting the vapors is illustrated in Figure 8.

Figure 8. Apparatus used for collecting volatile gases by fractional condensation
A stream of nitrogen is passed through a liquid manure sample contained in the 5-liter round-bottom flask carrying with it the organic vapors given off by the manure. The gas stream first passes through a trap immersed in ice water, where most of the water vapor present in the stream of gas is condensed. It then passes through two traps immersed in a dry ice-ethanol bath (-80°C) where the remainder of the water vapors and any carbon dioxide is removed. The third trap, immersed in liquid nitrogen (-196°C), consists of two collecting tubes, one of which is filled with glass beads to improve the efficiency of the condensing process. It is in this trap that the remaining volatile components are collected. Condensate from this final trap is transferred into the chromatograph for analysis by means of a special collector-injector tube illustrated in Figure 9.

Figure 9. The collector-injector system

The transfer tube used in this study was made out of 1/8" O.D. stainless-steel tubing shaped into a coil with a shut-off valve and a Swaglok fitting at both ends of the tube. A hypodermic needle and a syringe barrel were soldered to short lengths of 1/8" stainless-steel tubing so that they could be attached to either end of the transfer tube. Once the condensate was transferred to collector-injector coil, it could readily be injected into the chromatograph.

The condensate was transferred from the liquid nitrogen trap to the chromatograph as follows: The collector-injector coil was connected in series with the other
three traps and also immersed in liquid nitrogen. With the system remaining in
operation, the liquid nitrogen trap was removed from the liquid nitrogen and placed
in a warm water bath. On warming, the condensate quickly vaporized and traveled
to the collector-injector coil where it again condensed. With the collector-injector
still immersed in liquid nitrogen, the shut-off valves were then closed thus isolating
the condensed vapors from the remainder of the system. The hypodermic needle and
syringe barrel were connected to the tube and the needle was inserted into the
injector port of the chromatograph. The valves were opened on either end of the
tube, and the liquid nitrogen bath surrounding the collector tube was replaced by a
warm water bath. Immediately, the condensate vaporized and flowed into the
chromatograph.

Although not used extensively in this study, this method is relatively simple.
It has the advantage of providing a truly typical sample of the volatile vapors without
possible contamination by reagents used in other concentration techniques.

**Concentration by Selective Absorption**

Selective absorption is still another method that can be used for the concentra-
tion of gases. This technique is useful as a means of identification and will be dis-
cussed more in the following section.

**Identification of Gaseous Components**

Selective absorption techniques use reagents in which specific chemical groups
are very soluble or which react to form compounds that can be collected in a concen-
trated form. Four chemical groups were collected and concentrated by this technique;
alcohols, amines, carbonyls, and sulfur derivatives. Each procedure is discussed
separately.
This is a modification of the procedure developed by Suffis and Dean (62) based on the qualitative separation of alcohols from other components by a non-aqueous extraction. The procedure involves bubbling nitrogen through a liquid manure sample in a 5-liter round-bottom flask and thence through four tubes, each containing 25 ml. of reagent. The first tube containing 1.2N HCl acid, removes the amines, while the second and third tube containing HgCl (3% W/V) and HgCN (4% W/V), respectively, removes the sulfur containing compounds. The fourth tube contains propylene glycol which has a high absorptivity for alcohols. Carbonyls, esters, and hydrocarbons are also absorbed by the propylene glycol but in lesser amounts.

After the volatile gases bubble through the reagents tubes for a period of 24 hours, the propylene glycol tube is removed and mixed vigorously with an equal volume of carbon tetrachloride and placed in a separatory funnel forming two layers. The lower layer is carbon tetrachloride; the upper layer is propylene glycol. The carbonyls, esters, and hydrocarbons are considerably more soluble in CCl₄ and as a result a high percentage of these compounds will be removed from the propylene glycol layer, leaving only the alcohols. The procedure is repeated twice, then the washed propylene glycol is placed in the distillation apparatus illustrated in Figure 10.

Figure 10. Apparatus used to distill alcohols from the propylene glycol
The propylene glycol is carefully heated to 150° C. At this temperature, the water and alcohols vaporize leaving the propylene glycol. The condensate, collected in an ice-bath, is then injected into the chromatograph for analysis.

**Carbonyls**

A simple and rapid method for the collection and separation of a wide variety of carbonyl compounds by gas chromatography is described by Hunter and Walker (63). The method consists of three steps:

1. Formation and separation of liquid or solid semicarbazone derivatives of carbonyl compounds
2. Regeneration of carbonyls from semicarbazones by addition of acid.
3. Separation of regenerated carbonyls by gas chromatography.

The carbonyls are collected using the same absorption train as that for alcohols except that the propylene glycol is replaced by dichloromethane. During collection the tube is cooled in an ethanol-dry ice bath. After the vapors have been collected, 1 gram of semicarbazide hydrochloride and 1.5 grams of sodium acetate are added to the mixture. The mixture is stirred 24 hours at 5° F. in a closed container using a magnetic stirrer. The dichloromethane extract is then separated in a separatory funnel and the aqueous portion extracted 4 times with 10 ml. aliquots of dichloromethane. Washings and the original dichloromethane extract are combined and filtered through a filter paper containing 0.50 gram of anhydrous sodium sulfate. The clear, colorless filtrate is evaporated to near dryness under a stream of air, at room temperature. The residue is then placed in a vacuum desiccator at room temperature and evacuated overnight by means of a mechanical vacuum pump. The oily residue finally obtained is redissolved in a minimum amount of dichloromethane. The mixture is transferred to a smaller container and again evaporated to dryness. A portion of the final,
essentially odorless residue is transferred to a 2 ml. Erlenmeyer flask containing a small glass bead. As a final step, 0.5 ml. of acid reagent \( (a) \) is added, the flask stoppered, shaken vigorously, and the solution injected into the chromatograph for analysis.

**Sulfur-containing Compounds**

The volatile sulfur compounds were concentrated by precipitating the sulfur derivatives as mercuric salts. This is accomplished by bubbling the volatile gases through a tube containing 25 ml. of mercuric chloride (3% W/V) followed by a tube containing 25 ml. of mercuric cyanide (4% W/V). The white precipitate from the mercuric chloride and the black precipitate from the mercuric cyanide are mixed together and treated with 8N HCl to release the sulfur-containing materials in the form of their original volatile gases. The apparatus for regenerating the gases is illustrated in Figure 11.

![Figure 11. Apparatus used to regenerate the volatile sulfur compounds](image)

\( (a) \) The acid reagent was prepared by diluting 3 ml. of 85% phosphoric acid to 25 ml. with water.
Acid is added to the precipitate by means of the funnel and the regenerated volatiles then passed through a distilled water trap to remove the HCl vapors from the sample. The gases are removed from the water trap with a syringe and injected into the chromatograph.

Amines

The amines were collected by bubbling the volatile gases through a reagent tube containing 25 ml of 1.2N HCl and forming the hydrochloride salts of the amines. The original gases were regenerated by adding NaOH to the reagent tube until the solution was neutral. The solution was heated to drive off the dissolved amines which were collected by means of a syringe. The decomposition products of HCl derivatives were then subjected to gas chromatography for further analysis.
RESULTS

Qualitative Chemical Tests

Qualitative chemical tests were performed on the atmospheric samples collected above the manure pits according to the solubility method previously described in the section titled Identification of Gaseous Compounds. The results are summarized in Table 1. A total of six classes of compounds and a few individual compounds were found to be present in the hog manure volatiles. These compounds included alcohols, amines, amides, mercaptans, sulfides, disulfides, hydrogen sulfide and ammonia.

Table 1. Results of solubility tests on samples of the atmosphere in a swine building.

<table>
<thead>
<tr>
<th>Division Tested 1</th>
<th>Reagent or Method Used</th>
<th>Result of Test</th>
<th>Interpretation of Test (Compound Class Identified)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (water soluble)</td>
<td>Ferricyanide Test</td>
<td>Blue-Green (+)</td>
<td>Amides</td>
</tr>
<tr>
<td></td>
<td>N-N-dimethyl-naphthylamine</td>
<td>Rose (+)</td>
<td>Amides</td>
</tr>
<tr>
<td>B (HCl soluble, insoluble in H₂O)</td>
<td>Yellow Ring (+)</td>
<td>Yellow (+)</td>
<td>Amides</td>
</tr>
<tr>
<td></td>
<td>Hindsburg</td>
<td>Yellow (+)</td>
<td>Primary Amines</td>
</tr>
<tr>
<td></td>
<td>Rose (+)</td>
<td>Rose (+)</td>
<td>Secondary Amines</td>
</tr>
<tr>
<td></td>
<td>Purple (+)</td>
<td>Primary Amines</td>
<td>Tertiary Amines</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Silver-NO₃</td>
<td>White ppt (+)</td>
<td>Mercaptan</td>
</tr>
<tr>
<td></td>
<td>Na-Nitroprusside</td>
<td>Red (+)</td>
<td>Sulfides</td>
</tr>
<tr>
<td></td>
<td>Isatin</td>
<td>Green (+)</td>
<td>Mercaptans and Disulfides</td>
</tr>
<tr>
<td></td>
<td>Nitrochromic Acid</td>
<td>Blue (+)</td>
<td>Alcohols</td>
</tr>
<tr>
<td></td>
<td>Ceric Nitrate</td>
<td>Amber (+)</td>
<td>Alcohols</td>
</tr>
<tr>
<td>Collected by</td>
<td>Lead Acetate</td>
<td>Black (+)</td>
<td>H₂S</td>
</tr>
<tr>
<td>Condensation</td>
<td>Copper Sulfate Test</td>
<td>Blue (+)</td>
<td>NH₃</td>
</tr>
</tbody>
</table>

1 Division listing according to Cheronis (44).
In addition, mercaptans and sulfides were identified as their mercuric derivatives from formations of black and white precipitates in mercuric cyanide and mercuric chloride solutions, respectively. The identification of disulfides was confirmed when after bubbling the volatiles through a mercuric acetate solution a white precipitate was developed with the addition of sodium chloride. The method described by Marbach and Doty (54) gave a positive test for hydrogen sulfide and methyl mercaptan; the presence of these compounds also was confirmed by the bismuth nitrate test of Koren and Gierlinger (53).

The groups of compounds thus found by these qualitative tests to exist in the hog manure volatiles set the stage for the subsequent chromatographic analyses.

**Chromatographic Analyses**

A gas chromatogram of the hog manure volatiles concentrated by the salting technique and collected as head space gas is shown in Figure 12. Eighteen separate peaks resulted from the analysis. Chromatograms obtained after treating the samples with specific functional group reagents were compared with the control chromatogram of Figure 12 and also with chromatograms of known compounds. These results are shown in Figures 13, 14, and 15.

Figure 13 shows that seven peaks were eliminated or greatly reduced when the gas sample was treated with acidic hydroxylamine. This reagent reacts with and eliminates all carbonyl compounds thus identifying the eliminated peaks as either aldehydes or ketones.

In Figure 14, three of the original peaks are eliminated but reappear as three sharply eluted peaks in the beginning of the chromatogram after treating the sample with sodium nitrite solution. This treatment converts the alcohols to their respective
CHROMATOGRAM SHOWING THE ANALYSIS OF SWINE ODORS IN CONFINED HOUSING

- Oven Temp.: 65°C
- Detector Temp.: 50°C
- Injector Temp.: 140°C
- Nitrogen Flow Rate: 30 ml/min.
- Hydrogen Flow Rate: 30 ml/min.
- Sample Size: 0.5 ml, 2% Acid Gc
- Column: 6 ft x 1/8" Stainless Steel, 2% Carbowax on Chromosorb P

Figure 12. A chromatogram of the hog house odors concentrated by the salting technique

nitrates and thus identifies the alcohols.

Figure 15 gives a semi-log plot of retention time vs. carbon numbers for known compounds of n-alcohols and aldehydes. For comparison, the retention times for the alcohols and carbonyls identified in Figures 13 and 14 are plotted on this graph and designated as numbers corresponding to the peak numbers on the control
Figure 13. A chromatogram of a sample of hog manure volatiles after treating the sample with acidic hydroxylamine.
Figure 14. A chromatogram of a sample of hog manure volatiles after treating the sample with a sodium nitrite solution.

The alcohols were also collected by selectively absorbing the alcohol fraction from the volatile gases through a sodium nitrite trap. A chromatographic analysis of the reagent showed that five alcohols were absorbed as illustrated in Figure 16. Sulfuric acid (1 N) was then added to the reagent trap to convert the alcohols to their nitrite derivatives. The results of this reaction are shown on the chromatogram.
Figure 15. A semi-log plot showing the retention time vs. carbon numbers for n-alcohols and aldehydes of Figure 17. The retention times of the peaks eluted in Figure 16 were compared with the retention times of known mixtures of both normal and iso-alcohols. These data are shown in Figure 18. The retention times of the known alcohols were used to establish the straight line. The points, corresponding to the peak numbers on the original chromatogram, have as their x-coordinate a whole number that lies on the straight line, thus providing a positive identification of the eluted peaks.
Figure 16. A chromatogram showing the alcohol fraction of hog manure volatiles collected in a sodium nitrite reagent.

A chromatogram showing carbonyls regenerated from their semicarbazones is presented in Figure 19. These carbonyls were collected and concentrated from the manure odors as described in the section titled Concentration by Selective Absorption. The individual components were identified by comparing the

Figure 17. A chromatogram of the alcohol fraction after conversion to nitrites by a sulfuric acid treatment.
retention times to those of known carbonyls. Figure 20 shows the chromatogram of known carbonyls under the same operating conditions.

Alcohols were selectively absorbed by still another method. The manure volatiles were bubbled through a reagent tube containing propylene glycol. The alcohol fraction was then collected by a distillation procedure and injected into the chromatograph for analysis. The results of this technique are shown in Figure 21. Eleven peaks resulted from the analysis, seven of which were identified as alcohols from retention times of known alcohols. The chromatogram of the known alcohols under the same conditions is illustrated in Figure 22.
Figure 19. A chromatogram of carbonyls collected as derivatives of semicarbazones.
Figure 20. A chromatogram of a known mixture of carbonyls
Figure 21. A chromatogram of alcohols collected in a propylene glycol trap.
The volatile sulfur compounds of the manure collected as their mercuric derivatives were regenerated by an acid decomposition of the mercuric precipitate. An analysis of the regenerated gas sample yielded seven peaks as shown on the chromatogram of Figure 23. These sulfur compounds were composed of mercaptans, sulfides, and disulfides. However, no attempts were made to further identify the peaks.
Figure 23. A chromatogram of the sulfur volatile gases regenerated from their mercuric derivatives.

In summary, the solubility tests together with chromatograms have provided positive identification of a number of chemical classes in the composition of a hog house atmosphere. Classes identified as being present were amines, amides, alcohols, carbonyls, disulfides, sulfides, and mercaptans.
DISCUSSION OF RESULTS

The results of this study indicate that seven homologous groups, containing twenty-seven identified compounds, have been found in the volatiles given off from hog manure. These gases can be found in the section titled Results and are included in Table 1 and Figures 12 through 22. The homologous groups were selectively collected and subjected to chromatographic analysis for separation and identification of the individual components, following their qualitative identification according to solubility classifications.

Concentration techniques were used to prepare the samples, partially because of the limited sensitivity of the analyzing equipment, and also because of the minute quantities of the gases present in the hog house atmosphere.

Concentration Techniques

The three methods employed for concentrating the manure volatiles were: (1) salting, (2) selective absorption and (3) fractional condensation. The relative merits of these techniques, as used in this analysis, are discussed to enable a better evaluation of the results.

Salting Technique

Concentration of the volatile gases existing above a holding pit can also be accomplished by means of the salting technique. Enrichment of the head space gas above an aqueous solution is accomplished by the addition of anhydrous sodium sulfate to a vial containing the liquid sample. Advantages of the method are that it requires a minimum amount of equipment, is easily conducted, and can be accomplished in a matter of 8-10 minutes.
The eluted peaks of the head space gas can be readily identified by treatment of the liquid sample with selective qualitative reagents that eliminate homologous peaks. The head space gas can also be treated in the syringe to help qualitatively identify the volatile gases.

One disadvantage of this technique is that the sample must be heated to 60° C. for release of the dissolved gases from the aqueous solution. Heating alters the conditions under which the manure normally exists and may create a change in the amounts and types of gases. Although quantitative analysis can be carried out on the sample, the volatility of the gases at the elevated temperature must be carefully considered if this data is to be related back to normal hog house conditions.

Of the eighteen peaks eluted by this method, seven aldehydes and three alcohols were identified positively by their reaction with selective reagents (Figures 13 and 14) and their organization on a homologous series graph (Figure 15).

Peaks identified as (10 and 11) and (14 and 15) in Figure 12 were greatly reduced when treated with acidic hydroxylamine, indicating the presence of a carbonyl. These same peaks disappear when treated with sodium nitrite solution and produce instead the expected alcoholic nitrite peaks. This behavior indicates one of two things; either that in each case one alcohol and one aldehyde exist but the reaction for elimination is not complete, or that both an alcohol and an aldehyde exist at that retention time but are masked by two other compounds with similar retention times. In both cases, a positive identification of the presence of an alcohol and an aldehyde is made, as later borne out in Figure 15.

Samples collected by the salting technique also were treated with sodium bicarbonate and basic hydroxylamine to test for the presence of organic acids and esters, respectively. In both tests, the results were negative. Because of the
strongly basic nature of the material in the holding pits (pH of 8.0 or higher), volatile acids would not be expected to be present in a hog house atmosphere. The basic condition would also account for the negative tests for esters as their formation is based on the combination of acids with alcohols.

Selective Absorption

Of the three concentrating methods, the selective absorption technique appears to be the most reliable. In this method, a stream of nitrogen is bubbled through the manure sample carrying the volatiles to the various reagent traps where they are selectively absorbed. This procedure allows the volatile components to be collected without altering the conditions under which the manure normally exists.

The volatiles, once collected in their corresponding reagent traps, can then undergo additional treatment to further concentrate the gases. A gas sample, in its concentrated form, provides a higher detection limit and therefore yields a more complete analysis.

Another advantage of this technique is the ease with which samples may be injected into the chromatograph. Once the volatiles are absorbed by the traps, there is little danger of contamination from the surrounding atmosphere. In addition, the volatiles become quite stable in their collected form, with little possibility of escape and re-entry into the atmosphere.

This technique, by its selective nature, also facilitates identification because the eluted peaks represent functional groups. Thus, the collected volatiles can be readily identified by means of retention time.

This method further lends itself to quantitative measurements of the volatile gases by accurate measurement of the system flow rate. The volume of gas bubbled through the reagent tube, coupled with quantitative measurements of the concentrated
gas peaks, permits computation of the concentrations of the gases that exist in the hog house.

The selective absorption of the volatiles belonging to a given homologous group was another effective method used to concentrate the gases. Reagent traps were used to collect amines, alcohols, carbonyls, and sulfur volatiles.

Reagent traps containing sodium nitrite and propylene glycol were equally successful in collecting alcohols (Figures 16 and 21) and provided confirmation of similar findings by the salting technique.

Methanol, n-propanol and n-butanol were detected in all three concentrations techniques. Although the presence of the additional peaks in Figures 16 and 21 could be due to the differences in age and strength of the manure, it is believed this primarily indicates the relative effectiveness of the concentrating techniques.

The selective absorption methods generally are more effective than the salting technique simply because a larger volume of the atmosphere is employed for the collection of the sample. Of the two selective reagents, propylene glycol is the more effective for two reasons; it has a high absorptivity for alcohols and it has a high boiling point which permits the absorbed alcohols to be further concentrated by distillation.

The presence of carbonyls was confirmed by selectively absorbing them as their semicarbazone derivatives, shown in Figure 19. Previous qualitative tests for carbonyls on the manure volatiles, collected according to their solubility classifications, had yielded negative results. This can be attributed to the low sensitivity of the test reagent (2, 4 - Dinitrophenylhydrazine) used for detection.
Fractional Condensation

This method, like that of selective absorption, is performed without altering the conditions under which the manure exists. The main disadvantage with this method is the difficulty involved with transfer of the condensed gases to the chromatograph. Identification of the eluted peaks can be aided by treating the sample with selective qualitative reagents. Again, the disadvantage here, is the difficulty of handling the sample.

Unless a good procedure is employed to deal with the sample transfer, there is a considerable chance of sample contamination or of loss of much of it to the atmosphere.

Atmospheric Components

The results show that a hog house atmosphere contains many organic volatile compounds which fall into several homologous groups. Briefly repeated, the functional groups found were amines, amides, alcohols, carbonyls, mercaptans, sulfides, and disulfides. It is believed that the amines, mercaptans, sulfides and disulfides, resulting from the breakdown of the amino acids, constitute most of the objectionable odors from decomposing manure.

Body odors along with respired air perhaps account for a portion of the odors in an enclosed hog house. However, these odors are negligible by comparison with those from fresh and decomposing manure.

A study of the biological degradation of organic matter shows that several of these basic groups can be reasonably expected as intermediary compounds in the metabolic breakdown. The numbers and types of gases depends on many things, including the age and strength of manure, the age and size of the animal, the manure
management system in the building and the ration fed to the animals.

The original source and concentrations of these gases are other important considerations. Fresh excreta deposited on the floors by the animals, undoubtedly accounts for a great portion of the odor. Many of the higher organic volatiles, resulting from bacterial and enzymatic action within the animal, come from this source. As degradation of the excreta progresses, lower volatile compounds are created as a result of the many metabolic processes. Organic acids from the deaminization of amino acids, the glycolysis of carbohydrates, and the beta-oxidation of fatty acids are formed. These lower organic acids, eventually turn into methane and carbon dioxide, with aldehydes and alcohols formed as intermediate products.

Decarboxylation, coupled with deaminization of the amino acids, result in the production of amines, mercaptans, sulfides and disulfides, believed to be the cause of the objectionable odors.

Although no report has been made of the fixed gases (CO₂, CO, NH₃, SO₂, etc.) it should not be inferred that these gases are absent. Day et al. (4) have reported on the concentrations of these gases in relation to management, ventilation, and building parameters of an enclosed hog house. It is intended that such studies also will be included in future efforts in order to complete the long range objectives of this program.
SUMMARY

The objectives of this study were to identify those gases present in the atmosphere of a confined swine production system, other than those gaseous elements known to compose normal air. Known metabolic pathways of bacterial reduction of organic wastes indicated that several homologous groups of organic volatiles should be present in addition to the commonly fixed gases (CO₂, CO, H₂S, NH₃, SO₂, etc.) previously reported in the literature. Solubility tests together with chromatograms of the hog manure volatiles were prepared in this study, resulting in the positive identification of twenty-seven gaseous compounds.

The volatile gases were collected from liquid manure samples contained in holding pits beneath the slotted floors of the animal chambers in the AISI-ISU Swine Atmosphere Research Laboratory. To ensure that the existing volatile gases could be detected by means of gas chromatography in their normal combinations, various techniques were employed to enrich the gas samples before analysis. Selective qualitative reagents were used to establish the functionality of the chromatographic components which, when coupled with homologous plots and retention data, provided a positive identification for each gas.

As summarized on the following page, the groups of gases identified as being present were amines, amides, alcohols, carbonyls, disulfides, sulfides, and mercaptans and the fixed gases, methane, ammonia, and hydrogen sulfide. It is believed, from the previously known nature of the above compounds, that the amines and sulfur volatiles are responsible for creating most of the objectionable odors in an enclosed hog house. It is further believed that the major portion of the gases that constitute a hog house odor are identified. The functional groups detected were,
volatile sulfur compounds;
   mercaptans, sulfides, disulfides

volatile nitrogen compounds;
   amines, amides

and volatile carbon compounds;
   alcohols: methanol, ethanol, n-propanol, iso-propanol, n-butanol,
   iso-butanol, iso-pentanol
   carbonyls: formaldehyde, acetaldehyde, propionaldehyde,
   iso-butilaldehyde, valeraldehyde, heptaldehyde,
   octaldehyde, decaldehyde,

The value of this study then, although it is not complete in the sense of the
detection and identification of all the gases present in a hog house, is that it does
provide a successful starting point for the needed additional studies. It demonstrates
some techniques through which the atmosphere in an animal production building can be
explored, and it expands on previous literature which heretofore has reported only on
fixed gases. Future studies relating to the concentrations of the identified gases with
variations in the age and size of the animal, the age and strength of the manure and
management practices, should make it possible to evaluate the important economic and
health implications.
SUGGESTIONS FOR FURTHER RESEARCH

The comprehensive objectives of the research at Iowa State into the composition of the atmosphere in a livestock production building are to provide new information needed to complete a current engineering assignment. This assignment is to establish the design criteria for facilities to safely and economically rear animals in confinement. To complete the original objectives, and in the light of the experience and insight gained through this study, the following studies seem needed.

1. Additional tests to investigate for additional gases that might exist in a livestock building. It is believed that the most logical approach would be via new and more sensitive concentrating techniques thus utilizing the available equipment.

2. Studies to assess the effects that various concentrations of these gases, both singly and in mixtures, will have on the physiological and physical behavior of animals and materials, respectively. The effects of aerosols in combination with these gases also will need to be considered.

3. Once the economic implication of the toxic gases are evaluated, studies to investigate means of controlling their production. This will involve determining their sources along with their rate of production under varying environmental conditions.


ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Thomsen E. Hazen for his guidance and suggestions throughout the course of this study and the preparation of this thesis.

The author extends his thanks to Mr. Donald Baker and his staff for their assistance in the operation of the test facilities, to Dr. Duane W. Mangold and Mr. Arthur R. Mann for their advice and to the American Iron and Steel Institute for their support in this study.
Appendix A. Abstract of the Initial Report Submitted to the Members of the Committee of Galvanized Shear Producers Describing the Building Design and Exposure Tests
THE BUILDING

This report describes the laboratory which was placed in operation in November 1965, and the tests to be conducted within. The building erected on the Swine Nutrition Experimental Station of Iowa State University, is of the rigid-frame type, 42' long by 30' wide, with the exterior siding and roofing and the interior wall and ceiling surfaces of ribbed vinyl coated steel panels. The walls and ceiling are insulated with a glass fiber blanket with a total resistance (R value) of 8.0. A one-inch polystyrene board provides a perimeter insulation for the building floor. Figure 1a shows details of the construction and 1b a floor plan.

Each animal test chamber is 19' - 6" long, 14' - 4 1/2" wide, and 8' - 0" high, contains three pens which are 15' long and 4' wide; and each pen accommodates 8 pigs. The laboratory is a 16' - 0" X 8' - 0" room. The remainder of the building (about 8' X 22') houses the mechanical and control equipment and gives access to the chamber.

THE TEST CHAMBERS

The three pens in each test chamber are identical with respect to their construction and arrangements.

The Flooring System - The floor system in these units was designed to be flexible with regard to obtaining type of flooring; i.e., solid, slotted, or a combination of both, Figure 2c. A subfloor or pit, 4' - 0" wide, is located lengthwise underneath each pen, Figure 2b; each pit begins with a 1' - 0" stepdown at the north end, extends southward for 11' - 3 1/4" at a slope of 1/2" per foot, has another 0' - 4" stepdown and continues its slope for another 3' - 4". A deep narrow gutter, common to all three pits runs widthwise at the south end, is 6" wide at its lowest point, 8" at the
NOTE: ALL SLOTTED FLOOR PANELS ARE REMOVABLE. ALL PENS MAY BE USED AS
A. SOLID FLOOR
B. PARTIALLY SLOTTED
C. TOTALLY SLOTTED

FLOOR SLOPE
½" PER FOOT

ANIMAL AREA

TYPICAL SECTION
SCHEMATIC OF THE FLOORING SYSTEM

Figure 20
LONGITUDINAL SECTION

FIGURE 3a

TRANSVERSE SECTION

FIGURE 3b

FLOOR SECTIONS IN PEN AREA
top and has a minimum depth of 3'-6" and a maximum depth of 4'-4" (See Figures 3a and 3b).

A steel framework (Figure 2c) provides a support system to elevate the flooring materials to the desired level. This framework permits various floor slopes by interchanging supports at the front of the pit.

The initial floor in each pen is a 12'-0" length of solid flooring with a 3' - 0" section of slotted flooring over the deep narrow gutter. The solid flooring section is made of concrete slabs 2' - 0" wide, 4' - 0" long and 2" thick, bound by a sheet metal form. Each slab is reinforced with #10/10, 6" X 6" wire. The sides and bottom of each slab are coated with pitch to prevent corrosion of the metal form.

The slotted floor sections were fabricated in the Republic Steel Corporation's Research Center from materials supplied by the various cooperating steel producers. The panels measure 4'-0" by 4'-6" and contain elements of eleven different analyses of steel as shown in Figure 4.

The panel distribution for each of the three years that the panels will be exposed was prepared by Mr. K. F. Below of the Republic Steel Corporation (Table 1). Panels 1, 2, 3, 4, 5, and 6 will be exposed the first year in the pens indicated. Panel exposure for the second and third year will include panels 1, 2, 3, 7, 8, and 9. Thus, panels 4, 5, and 6 will be exposed for one year; panels 7, 8, and 9 for two years and panels 1, 2, and 3 for three years. All panels will be returned to Republic Steel to be analyzed by Dr. Kowalski.

Pen Partitions - The pen partitions, composed of six types of steel materials were developed and installed under the supervision of Mr. C. H. Lawson of the Armco Steel Corporation. Fabricated by the Clay Equipment Corporation, the pen work was constructed of 3/4" galvanized pipe (Figure 5) so that the steel panels could be easily mounted to the frame. Clamp bands mounted on the frame and fastened "by 1/4" x 1/2"
Table 1

STEEL SLOTTED FLOOR TEST PANELS

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Material</th>
<th>Condition</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cor-Ten T</td>
<td>Assem. as shown</td>
<td>USS</td>
</tr>
<tr>
<td>2.</td>
<td>Republic 50</td>
<td>Blast Cleaned</td>
<td>RSC</td>
</tr>
<tr>
<td>3.</td>
<td>Porcelain on steel</td>
<td>As H. R.</td>
<td>ASC</td>
</tr>
<tr>
<td>4.</td>
<td>H. D. Galvanized</td>
<td>Epoxy Coating</td>
<td>USS</td>
</tr>
<tr>
<td>5.</td>
<td>H. D. Galvanized</td>
<td>USS</td>
<td>USS</td>
</tr>
<tr>
<td>6.</td>
<td>Republic 50</td>
<td>As H. R.</td>
<td>RSC</td>
</tr>
<tr>
<td>7.</td>
<td>Modified 410 grade</td>
<td>RSC</td>
<td>RSC</td>
</tr>
<tr>
<td>8.</td>
<td>Alphatized</td>
<td>USS</td>
<td>ISC</td>
</tr>
<tr>
<td>9.</td>
<td>Carbon</td>
<td>Rimmed</td>
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<tr>
<td>10.</td>
<td>Cor-Ten</td>
<td>Blast Cleaned</td>
<td>USS</td>
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<tr>
<td>11.</td>
<td>Cor-Ten</td>
<td>As H. R.</td>
<td>USS</td>
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DISTRIBUTION OF MATERIALS FOR EACH PANEL

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<thead>
<tr>
<th>Panel Number</th>
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<th>3</th>
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<td>3</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>9</td>
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<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>5</td>
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<td>11</td>
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<td>3</td>
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<td>10</td>
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</tr>
<tr>
<td>Distribution</td>
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<td>3</td>
<td>3</td>
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<td>9</td>
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<table>
<thead>
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<td>10</td>
<td>3</td>
<td>11</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>
PLAN

PLAN OF SLOTTED FLOOR

SECTION A

FIGURE 4
PARTITION

FRONT GATE

ELEVATIONS OF PANEL FOR GATE & PARTITION

FIGURE 5
machine screws are used to secure the steel panels to the frame. The steel panels are 6 1/2" x 24" and are mounted to the clamp bands by means of four 3/16" x 3/4" nylon bolts; spacer bars fastened to the clamp bands provide an even spacing of the steel panels on the frame. Each chamber contains six gates and eight panel sections. A set of six different steel plates (Table 2) form a sequence which is repeated in each pen with one set of six on each gate and two sets of six on each panel section.

Each set of steel plates contains a number which is stamped on the upper right hand corner of each plate. There are 44 sets of plates, arranged statistically throughout the test chamber (Figure 6). At the end of the first year of exposure, 22 sets of plates will be dismounted and returned to Mr. H. H. Lawson of Armco Research Laboratory for testing and analysis. These plates will be replaced by 22 other sets of plates and at the end of three years all the plates will be removed providing a two and three year exposure table.

Test Samples - Three test racks with duplicate coupons of 14 different metals are mounted in each test chamber. The responsibility for obtaining tests and analysis coupons rests with Mr. S. S. De Forest of the United States Steel Corporation. The coupons, 4" wide by 6" long, are held in place by insulators mounted on a stainless steel rack. The racks are fastened to the walls at a height of five feet from the floor to the bottom of the rack and arranged parallel with the direction of the pen partitions. The various metals are listed in Table 3.

At the end of the first year of exposure, one rack from each chamber will be returned to R. Smith of United States Steel Applied Research Laboratory for analysis. Two more racks, one from each chamber, will be returned at the end of two years and the remaining two racks will serve as three year exposure tests for the metal coupons.
Table 1 continued

PANEL EXPOSURE DISTRIBUTION

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<thead>
<tr>
<th>Pen</th>
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<th>6</th>
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<td>1st Year</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2nd Year</td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>3rd Year</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

1 yr. Panels 4-5-6
2 yr. Panels 7-8-9
3 yr. Panels 1-2-3

Table 2

Sequence of materials used for gate end panel partitions:

1. Porcelain on steel
2. Aluminum coated
3. Galvanized
4. Cor-Ten
5. Galvanized wire mesh
6. Modified 410 grade

Table 3

List of materials used for coupons on test panel:

1. Carbon steel (U.S. Steel Corporation)
2. Cor-Ten steel (U.S. Steel Corporation)
3. Republic 50 (Republic Steel Corporation)
4. Galvanized 2 oz. coating (Armco Steel Corporation)
5. Aluminum coated Type 11 (Armco Steel Corporation)
6. Modified 410 grade (U.S. Steel Corporation)
7. Type 301 stainless steel (Republic Steel Corporation)
8. Aluminum coated Type 11 (U.S. Steel Corporation)
9. Acrylic painted (Armco Steel Corporation)
10. Aluminum (Armco Steel Corporation)
11. Porcelain on steel (Republic Steel Corporation)
12. Alphatized (Inland Steel Company)
13. Cor-Ten -- blast cleaned (U.S. Steel Corporation)
14. Republic 50 -- blast cleaned (Republic Steel Corporation)
<table>
<thead>
<tr>
<th>EAST ROOM</th>
<th>WEST ROOM</th>
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<tbody>
<tr>
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<td>0</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

NORTH
THE VENTILATING SYSTEM

The ventilating system for providing variable but controlled quantities of constant-temperature air to the chambers consists of two heat exchangers; an air reservoir; a damper complex for each chamber which modulates so as to provide a constant temperature of the incoming air regardless of the outside conditions; and three, 3-speed fans in each chamber to draw air into the animal units.

The reservoir, with an average height of 6' - 0", occupies the space above the test chambers and alley way. All surfaces of the reservoir are insulated with a glass fiber blanket having a total resistance (R value) of 8.0.

Located in the air reservoir are two heat exchangers, each capable of delivering 160,000 btu/hr at their design load. These heat exchangers are connected to the split end of a Y-duct which delivers only outside air to the air reservoir, Figure 7.

The damper complexes (Figure 6) are also located in the air reservoir and control the ratio of the heated air within and the outside air which is ultimately delivered to the animal chambers. No air is recirculated from the test chambers to the reservoir.

The air enters the Y-duct, divides itself, passes through the heat exchanger and enters into each animal chamber. It meets a modulating damper which controls, by means of a sensing device in the test chamber, the final ratio of air from the reservoir and from the outside. Upon entering the animal chamber, the mixed air enters a duct containing three, 3-speed fans which distribute the air throughout the chamber (Figure 3). The air leaves the chamber by means of two 1' - 0" by 2' - 0" exhaust openings located opposite the intake fans.

A solenoid valve and spray nozzle, controlled by a humidistat in the animal chamber, is located in each modulating damper duct system to add moisture to the incoming air so that a minimum humidity level could be maintained in the test
chambers at all times.

This system provides a modest but fairly responsive control of the temperature, ventilation rates, and relative humidity in each chamber, the range of which will be dependent upon the outside weather conditions.

**THE HEATING SYSTEM**

The heating equipment consists of two 160,000 btu/hr., LP-gas fired, hot water boilers; a 1000 gallon LP tank; two heating coils; and a 15,000 btu/hr. LP-gas fired wall heater located in the laboratory (Figures 7 and 8).

Each boiler operates in conjunction with a heating coil as a separate system; however, the boilers are interconnected so that either one could operate both heat exchangers in case of break-down or maintenance problems. Pressure gages and thermometers are positioned on each system to provide the operator with adequate information about pressure and temperature of the water entering and leaving each boiler as well as each heating coil. Unions and valves throughout the heating system allow one to isolate any part of the system for repair without shut-down of the remaining system.

**THE ELECTRICAL SYSTEM**

The electrical system consists of 14 circuits (Figure 9). Water proof receptacles are spaced throughout the building to provide an adequate source of power for working convenience.

The five receptacles in the lab are separately circuitted. These receptacles were installed for use with analyzing and recording instruments which may drift from their true readings if not electrically isolated from other power demands.
FRONT ELEVATION OF VIEW "A"

DAMPER COMPLEX

FIGURE 8
THE DRAINAGE SYSTEM

A 4" cast iron pipe network (Figure 10) provides the necessary drainage requirements and means for collecting and sampling wastes. Floor drains are located in the lab, mechanical room, and in the center aisle.

A 90° elbow, located at the south-west corner of each animal chamber, connects the deep narrow gutter outlet to the cast iron pipe. The building drain then runs through the west side of the building, at a slope of 1/2" per foot, to a manhole, located 150 feet west of the building and then enters the lagoon.

THE LABORATORY

The laboratory contains a 15,000 btu/hr. LP-gas-fired wall heater; a 16,000 btu/hr. air-conditioner, and an exhaust fan to provide a constant-temperature environment at all times for the recording and analyzing equipment.

Two exhaust hoods are mounted on the north wall of the lab above the work table to collect the exhaust and excess accumulation of gases in the laboratory. Beneath the duct work (Figure 11) are panels for regulating the temperature and ventilation rates in each animal chamber.

The laboratory is also equipped with hot and cold water and is well lighted.
Appendix B. Description of the Operation of the Test Chambers
Operation of the Test Chambers

Animals were introduced into the chambers as weaned pigs of approximately 40 lbs. body weight, were self-fed, and removed from the building as soon as they reached market weight. Each chamber contained 24 animals, separated into three groups of 8.

The pens were hosed down as needed which at most was once per week during warm weather. The deep narrow gutters were emptied and flushed about once each three weeks.

The ventilation rate was the same in both chambers, varying up to about 1000 cubic feet per minute. The temperature during the winter months was maintained at approximately 65°F. During the summer months, the temperature varied diurnally at whatever equilibrium temperature the ventilation rate would permit. Relative humidity in both chambers ranged between 55 and 75 percent during the tests.
Appendix C. Special Chemical Reagents

Bismuth Nitrate Reagent (53)

Add 0.22 gms. of BiNO₃·5H₂O to 25 ml. of a 3.2% (W/V) mannitol solution.

After it dissolves, add 8 ml. of pure glycerine and 36 ml. of 2.5% gum arabic solution that had been previously filtered.

Add acetic acid - sodium acetate buffer bringing the mixture to 100 ml.

Age for 2 days and then filter the solution. The pH should read about 4.6.

(The Acetic acid - sodium acetate buffer is prepared by mixing 11 volumes of 0.2 molar sodium acetate with 3 volumes of 0.2 molar acetic acid.)

Note: Add an anti-foaming agent if the mixture is to be used in a bubbling setup.

Acid Amine Solution (54)

Dissolve 5.0 gms. of N₂N-dimethyl-p-phenylenediamine hydrochloride in 1 liter of concentrated hydrochloric acid. The solution should have an absorbance value of 0.03 or less at 500 μm with a 16-mm. path length. When protected from light the solution is stable indefinitely.

Reissner Solution (54)

Dissolve 67.6 gms. of ferrous chloride hexahydrate in distilled water, dilute to 500 ml., and mix with 500 ml. of a nitric acid solution containing 72 ml. of boiled concentrated nitric acid (specific gravity 1.42). This solution is stable indefinitely.
Appendix D. Analysis of the Manure and Feed Formulation

Table 2. Chemical analysis of a typical sample from the manure pit

<table>
<thead>
<tr>
<th>Nitrogen Analysis</th>
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<tbody>
<tr>
<td>Organic Nitrogen</td>
<td>1,340 mg/l</td>
</tr>
<tr>
<td>Ammonia Nitrogen</td>
<td>5,360 mg/l</td>
</tr>
<tr>
<td>Nitrite Nitrogen</td>
<td>265 mg/l</td>
</tr>
<tr>
<td>Nitrate Nitrogen</td>
<td>330 mg/l</td>
</tr>
</tbody>
</table>

Total Sulfur                       | 4,400 mg/l |
Volatile Acids                     | 33 mg/l    |
Total Solids                       | 785 mg/l   |
Volatile Solids                    | 26 mg/l    |

B.O.D.                             | 60,000 mg/l |
C.O.D.                             | 201,000 mg/l |
Table 3. Composition of Ration

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Pounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn (ground)</td>
<td>82.75</td>
</tr>
<tr>
<td>Solvent soybean oil meal (50% protein)</td>
<td>13.50</td>
</tr>
<tr>
<td>Vitamin premix</td>
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</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.90</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.25</td>
</tr>
<tr>
<td>Salt (iodized)</td>
<td>0.50</td>
</tr>
<tr>
<td>Trace mineral premix*</td>
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</tr>
<tr>
<td>Aurofac 40</td>
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</table>

Calculated Analysis

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</thead>
<tbody>
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<tr>
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</tr>
<tr>
<td>Phosphorous, percent</td>
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<td>Vit. A, I. U. per lb.</td>
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</tr>
<tr>
<td>Vit. D$_2$, I. U. per lb.</td>
<td>300</td>
</tr>
<tr>
<td>Riboflavin, mg. per lb.</td>
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</tr>
<tr>
<td>Ca pantothenate, mg. per lb.</td>
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</tr>
<tr>
<td>Niacin, mg. per lb.</td>
<td>18.2</td>
</tr>
<tr>
<td>Choline chloride, mg. per lb.</td>
<td>352</td>
</tr>
<tr>
<td>Vit. B$_12$, mcg. per lb.</td>
<td>10</td>
</tr>
<tr>
<td>Chlortetracycline, mg. per lb.</td>
<td>20</td>
</tr>
</tbody>
</table>

*Contribution per lb. ration: Fe, 70.4 ppm; Cu, 4.8 ppm; Co, 1.6 ppm; Zn, 82.6 ppm; Mn, 56.8 ppm.