NITRITE TOXICOSIS AND THE GASTRIC ULCER COMPLEX IN SWINE

PART I. NITRITE TOXICOSIS

PART II. GASTRIC ULCER COMPLEX

Thesis for the Degree of Ph. D.
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This is to certify that the

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ABSTRACT

NITRITE TOXICOSIS AND THE GASTRIC ULCER COMPLEX IN SWINE

By Laurnie W. Nelson

PART I. NITRITE TOXICOSIS

Four experiments were conducted using a total of 49 pigs to evaluate and study nitrite toxicosis in swine. Thirty pigs in 2 different experiments were given up to 500 p.p.m. nitrite in their drinking water for 11 weeks. No major differences due to nitrite were observed in the growth rates, dressing percentages, organ weights, or white blood cell, hemoglobin or packed cell volume values. In addition, serum and liver vitamins A and E values were not influenced by nitrite. No nitrite residues were found in the muscle tissue or vital organs. Higher levels of nitrite occasionally caused listlessness, slight cyanosis, and lethargy in pigs shortly after eating and drinking. No lesions attributable to prolonged nitrite ingestion were found on gross and histopathologic examinations. The incidence of gastric ulcerations was not increased by the prolonged nitrite ingestion.

Nineteen pigs in 3 different experiments were used to study acute nitrite toxicosis. The LD_{50} of nitrite orally for pigs was calculated as 30 to 32 mg./lb. body weight. Anemia had no effect on the LD_{50}. Signs of acute nitrite toxicosis were primarily those of anoxia and death was apparently due to respiratory failure. Hematological studies

during the 4 hours following nitrite dosing indicated a transitory leukocytosis and an absolute neutrophilia, lymphopenia and eosinopenia. Methemoglobin and serum nitrite-nitrogen (NO_2-N) values were not related to different hemoglobin values in pigs. Decreased diastolic, systolic, and pulse pressures and increased heart and respiratory rates were noted after injection of nitrite into the stomach. Gross lesions in acute toxicosis included brownish discoloration of the blood and tissues, cyanosis, and hyperemia of the visceral and peripheral blood vessels.

PART II. GASTRIC ULCER COMPLEX

The epizoology of the gastric ulcer problem was studied using 1071 slaughter-age pigs from the Swine Evaluation Station, Michigan State University Swine Farm, and outside sources. The study indicated 378 (35.3%) had keratinization of the stomach, 115 (10.7%) had ulcers in the esophageal region (hereinafter called esophageal ulcers), and 38 (3.5%) had fundic ulcers. The 170 pigs from the Swine Evaluation Station had the highest incidence of stomach lesions at slaughter. Although all the pigs were clinically normal, 39 of 115 with esophageal ulcers had free and clotted blood in their stomachs. The pigs subjected to the stresses associated with growth experiments and environmental and management changes had the highest incidence of stomach lesions. Keratinization of the esophageal region was characterized by a parakeratotic proliferation of the squamous epithelium. This appeared to precede necrosis and ulcer formation. The esophageal ulcers and fundic ulcers appeared as separate and unrelated entities.

No differences were noted in the incidence of stomach lesions in 97 pigs given rations varying in vitamin A and carotene content. The

research on stomach pH values indicated that the average pH value was 2.5 in the normal stomachs, 3.8 in those with keratinization, and 6.2 in those with esophageal ulcers. Thus, low acidity, rather than hyper-acidity, appeared to be related to the stomach lesions.

Candida albicans was frequently cultured from stomach lesions, but this organism was considered a secondary factor because ulcers could not be experimentally reproduced. Using 12 pigs, it was established that C. albicans was capable of producing lesions following intravenous, subarachnoidal, and oral routes of administration.

Twenty-four pigs were used in the study of prednisolone-induced ulcers. Daily injections of prednisolone caused a high incidence of stomach ulcers, increased gluconeogenesis, inhibited inflammatory responses, and caused a leukopenia with an absolute lymphopenia.

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PART I. NITRITE TOXICOSIS

PART II. GASTRIC ULCER COMPLEX

By

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PART I. NITRITE TOXICOSIS

PART 1. NITRITE TOXICOSIS

INTRODUCTION

For many years, nitrite toxicosis was characterized by an acute anoxia. More recently, reports have associated nitrites and nitrates with a more chronic syndrome. The relationship of nitrites and nitrates with a vitamin A interference syndrome led to a postulated etiology for the increased incidence of the gastric ulcer complex in swine.

Numerous reports during the past 10 years have indicated an increased nitrite level in water and plant food supplies. Relatively low levels were reported to cause nutritional interference syndromes. This raised questions as to the safety of nitrite as an additive in cured meat products and to the possibility of a nitrite residue in fresh meat foods.

The primary purpose of this research was to produce acute and chronic nitrite toxicoses in swine and to investigate a possible relationship to gastric ulcers. Attention was also focused on the clinical, gross, and histopathologic aspects of acute nitrite toxicosis. This information would be of value in verifying the LD_{50}, in determining the hematologic response to a toxic chemical agent, in correlating methemoglobin and serum nitrite levels, and in elucidating the metabolism of nitrite.

REVIEW OF LITERATURE

Properties of Nitrite

The Pharmacopeia of the United States (1960) describes potassium nitrite (KNO_2) as a whitish granular compound with a molecular weight of 85.11. It is readily soluble in water and forms a slightly alkaline, highly ionized solution. The American Meat Institute Foundation (1960) characterized the nitrite ion as a highly reactive ion which can serve as both a reducing and an oxidizing agent. Although it can be oxidized to nitrate by powerful oxidizing agents such as permanganate, it usually acts as an oxidizing agent as in the oxidation of hemoglobin to methemoglobin. The curing reaction in meats is described as myoglobin plus nitrite to form nitrosomyoglobin. Although tissue respiration has ceased in the exsanguinated animal, fermentation and respiratory enzymes continue to be active. During this period, glycogen is broken down, releasing lactic acid. In this acidic environment an equilibrium is established between nitrous acid and nitric oxide. Nitric oxide (NO) forms complexes with hemoglobin, myoglobin and several of the porphyrin-containing enzymes by attaching to the iron in the heme portion of the molecule. Since myoglobin is the most prevalent pigmented compound in meat, some of the nitric oxide combines to form nitrosomyoglobin. This red compound is relatively unstable and therefore must be heated to form nitrosohemochrome, which gives the characteristic pink color to cured meat.

In contrast to the above chemical reaction in meat, Nason (1962) discussed how the nitrite ion is reduced by microorganisms into different intermediate and end products. From the +3 oxidative state of nitrite

to the -3 oxidative state of ammonia, the nitrogen atom passes through the following forms: +2 nitric oxide (NO); +1 nitrous acid (N_2O), nitroxyl (HNO), hyponitrous acid (H_2N_2O) and nitramide ($NO_2 \cdot NH_2$); 0 nitrogen (N_2); -1 hydroxlamine (NH_2OH) and -2 (NH_2NH_2).

Winter (1962) studied nitrite metabolism in cattle. Although nitrite was administered by drenching, both hydroxylamine and nitrite were demonstrated in the blood serum. Hydroxylamine was formed from the nitrite and was capable of converting hemoglobin to methemoglobin. Blood ammonia values were not influenced by administration of either nitrate or nitrite.

Pharmacodynamics

Beckman (1958) discussed the use of sodium nitrite in the treatment of heart conditions in man. The breakdown of nitrite in the acid medium of the stomach releases nitrous acid, which is irritating to the mucosa. It was assumed, but not proven, that the nitrite was largely decomposed in the gastrointestinal tract. About 70% of the absorbed nitrite disappears in the body, probably through conversion to ammonia. The remainder is excreted in the urine as nitrites and nitrates.

Nitrite relaxes the smooth muscles of all blood vessels, independent of their innervation. Due to this relaxation, the most striking pharmacodynamic effect is a fall in blood pressure. Because of the diminished venous return to the heart, the stroke volume decreases but a compensatory increase in heart rate preserves cardiac output. Toxicosis in man is characterized by a well maintained diastolic pressure and a low systolic pressure, which results in a small pulse pressure. This diastolic pressure indicates that "nitrite shock" is not due to the pooling of blood in the

postarteriolar vascular bed but is due to the venous pooling in the lower extremities and diminished venous return to the heart.

Serum Nitrite Determination

Diven et al. (1962b) suggested that total nitrate blood values were a better indication of the amount of nitrate consumed by sheep than methemoglobin values. Their research indicated that the nitrate blood values were highest about 2 hours prior to the outward signs of intoxication. They (1962a) also devised a rapid method for the quantitative estimation of serum nitrite- and serum nitrate-nitrogen. These methods were described for ruminant blood, and they indicated that a high degree of accuracy for nitrite analysis was possible.

Methemoglobinemia

Finch (1948) characterized methemoglobin ($HbFe^{+++}OH$) as a derivative of hemoglobin in which the ferrous porphyrin complex was converted to the ferric form. Since oxygen cannot combine with the ferric form, methemoglobin is of no value in respiration. In the intact red blood cell, methemoglobin is formed continuously, but it is also promptly reduced to the functional ferrous form through action of reducing agents such as ascorbic acid and glutathione which are normally found in the blood.

Huennekens et al. (1957) reported that the effectiveness of ascorbic acid and glutathione in relieving methemoglobinemia was independent of methemoglobin reductase and depended solely on the fact that these were reducing agents. However, methemoglobin reduction was accomplished by another methemoglobin reductase which depended on triphosphopyridine

nucleotide, generated by way of the "hexose monophosphate shunt". This enzyme required for its activity an added electron carrier for the normal reduction of methemoglobin. Substitution of this electron carrier by methylene blue explains the action of this dye in the treatment of methemoglobinemia.

Nitrite Toxicosis in Swine

Sources of Nitrites

Whey. Wanntorp and Swahn (1953) recognized nitrite toxicosis in swine fed byproducts of the cheese-making industry. Sodium nitrate was added to milk in a cheese-making process to prevent certain bacterial fermentations. When nitrate was reduced to nitrite, it inhibited the growth of some bacteria. They reported the LD_{50} of sodium nitrite for swine as below 90 mg./kg. of body weight.

Stormœrken (1953) noted that swine were more sensitive to sodium nitrite than cattle and sheep. He postulated the minimum lethal dose for swine to be 70 to 75 mg./kg. of body weight.

Cooked mangels and mangolds. Robinson (1942), in New Zealand, reported the death of 200 pigs of all ages in a herd of 600 which were fed freshly pulled mangels that were cooked about 2 hours the previous evening. The pigs died $\frac{1}{2}$ to 2 hours after the morning feeding. The post-mortem finding of methemoglobinemia was typical of nitrite poisoning. He also indicated that mangel poisoning in cattle was associated with the formation of nitrite from nitrate.

Also in New Zealand, McIntosh, Nielson and Robinson (1943) experimentally produced nitrite poisoning in pigs by feeding boiled mangolds:

1 to 3 Gm. $NaNO_3$ in mangolds per day to pigs of about 20 kg. in weight produced signs of poisoning, but they recovered.

Brine. In Canada, Gwatkin and Plummer (1946), concerned over salt poisoning in swine, found that pickling brine that contained potassium nitrate and nitrite was more toxic than other potassium salts. Under certain conditions the nitrate was reduced to nitrite by bacterial action and 500 to 800 p.p.m. of nitrite were demonstrated in some pickled products. While oral doses of 30 to 90 Gm. KNO_3 or 5 Gm. KNO_2 were lethal for small pigs (20 to 45 pounds), these dosages caused only a transient methemoglobinemia in pigs weighing 70 pounds. The only post-mortem finding was methemoglobinemia.

Herring. Hvidsten (1955) reported the use of sodium nitrite as a method of preserving factory herring, since herring were used as a protein supplement in swine rations. Feeding 200 Gm. per animal per day of a herring meal which contained up to 2% sodium nitrite had no influence on methemoglobin values in the blood, feed efficiency, or quality of the pork. When the herring meal contained 4% nitrite, and cod liver oil was mixed into the feed, no vitamin A was deposited in the liver. He postulated that the vitamin A was destroyed by contact with cod liver oil and nitrite.

Plant sources. Laing and McIntosh (1947), in New Zealand, indicated that sudden deaths in cattle and pigs were recorded occasionally during the previous 12 years. The signs included staggering, rapid breathing, convulsions, and death within an hour. They also indicated that animals could eat sublethal

amounts of nitrites daily without suffering harmful effects. No resistance was built up by the animals; the same dose would kill an animal that had been on a ration containing nitrite or an animal on a nitrite-free ration. Besides methemoglobinemia, they described a dilatation of the blood vessels throughout the body and an increased heart rate. Death was attributed to suffocation.

Case (1954) reported abortion in a drove of sows pastured on rape and oat pasture. Although some sows aborted 2 weeks before full term, others at full term had weak or dead pigs. The nitrate content of the rape was 5.52% and of the oats 0.53%.

Smith, Lovell, Reppert, and Griswold (1959) were among the first to report nitrate poisoning in pigs in the veterinary literature. The most prominent signs were labored breathing, loss of appetite, polydypsia, and convulsions. Losses were attributed to the pigs' eating damp straw, since when the straw was removed death losses stopped. Toxicity of the straw was explained on the basis of the reduction of nitrate to nitrite in the wet straw. The lesions found at necropsy included acute gastritis, edema of the lungs, conjunctivitis, and purplish discoloration of the abdomen.

Wunder and Schultz (1960) described nitrate poisoning in pigs caused from eating lambsquarter (Chenapodiun album) and redroot pigweed (Amaranthus retroflexus). The signs were rapid breathing and watery diarrhea. Necropsy revealed dark-colored blood, perirenal edema and subcapsular ecchymotic hemorrhages in the kidneys.

Water. Winks, Sutherland and Salisbury (1950), in Queensland, reported heavy losses on 2 farms where pigs were fed soup which was prepared by cooking beef and offal in well water. Since the nitrate content of the well water were 2970 and 1740 p.p.m., the mortality of the pigs was thought to be due to nitrite poisoning. These workers ascertained that 40 to 80 mg. $NaNO_2$ orally per kg. of body weight caused increased methemoglobin values and that 90 mg./kg. of body weight killed the pigs within 2 hours.

Bjornson et al. (1961) described outbreaks and sources of nitrate intoxication in cattle and swine in North Dakota. From July, 1959, to July, 1960, 47 cases of nitrate poisoning were diagnosed by the North Dakota State University workers. On one farm where swine losses occurred, the water from 4 wells contained 480, 510, 200 and 205 p.p.m. KNO_3 equivalents. Methemoglobinemia was a constant finding at necropsy of the animals. These authors also indicated that nitrates in the diet depleted the amount of vitamins A, D, and E.

Case (1963), in Missouri, reported serious losses of pigs on many farms. One outbreak involved pigs in an SPF program in which the extreme susceptibility of young animals to nitrite toxicosis was observed. Although nitrite toxicosis can be fatal, Case described a nutritional interference syndrome due to the nitrites which resulted in poor weight gains, diarrhea, and generally unthrifty animals. He also emphasized the importance of having water supplies analyzed for nitrates. For instance, on one farm the well contained 250 p.p.m., and the pond 30 p.p.m., KNO_3 equivalents, whereas the drainage ditch contained little or no nitrates. On the basis of his clinical observations in swine, he considered water with 30 to 50 p.p.m. nitrate-nitrogen (NO_3-N) as risky

and water with 50 to 200 p.p.m. NO_3-N and over as possibly causing heavy acute death losses. He also reported that nitrites were at least 10 times as toxic as nitrates.

Contaminated concentrates. Recently, Bush and Matteson (1963) described what they believed to be nitrate toxicosis in cattle and swine in Indiana. In swine the average mortality was 10 to 15%, but in some droves reached 90%. Chronicity with this problem resulted in unthriftiness and sporadic deaths. Although methemoglobinemia was not found, the necropsy findings included gastroenteritis, gastric and duodenal ulcers with free blood in the lumen, varicolored livers with areas of necrosis, ascites, subcutaneous edema, and pulmonary emphysema. These authors suggested that dicalcium phosphate with 20,000 p.p.m. nitrate and soybean meal with as high as 73,500 p.p.m. nitrate were the chief sources. Removal of the animals from rations high in plant-origin protein and replacement with rations high in animal-origin protein stopped the losses on most farms.

Nitrite Toxicosis in Rats

Pathology

Hueper and Landsberg (1940) described pathologic changes in the organs of rats produced by chronic nitrite poisoning. One of their experiments was devoted to sodium nitrite with 0.5 Gm. of $NaNO_2$ being added daily to a meat ration for each rat. This experiment began with 6 rats, 2 of which died during the first 2 weeks and 2 more of which were killed after 4 weeks. The other 2 rats were maintained on this diet for 18 weeks. Clinical signs included cyanosis, listlessness, and a marked loss of weight. The necropsy findings included brownish

discoloration of the blood and lungs, a soft dark liver, dark red kidneys and coffee-ground-colored mucosal erosions in the stomach. Histopathologic examination revealed degenerative vascular and parenchymatous lesions in the heart, liver, brain, kidney and testes. As an explanation for the development of these lesions, they concluded that nitrite caused vasodilatation which in turn caused a slowing of the blood flow and a stagnant hypoxemia.

Interference with Vitamin A Nutrition

Garner et al. (1958) reported a depletion of vitamin A in rats and swine fed 2% KNO_3 in the ration. O'Dell, Garner and Muhrer (1960), while studying the effects of nitrate in the rations of rats, noted a rapid depletion of vitamin A and the precipitation of a vitamin E deficiency.

Smith (1961) found that nitrite toxicosis was not restricted to methemoglobinemia but involved other oxidative processes. Since vitamin A and carotene are susceptible to oxidative destruction, it was conceivable that nitrite oxidized and destroyed vitamin A in the tissues. Smith postulated that in chronic cases of nitrite toxicosis protein enzymes necessary for conversion of carotene to vitamin A were altered and this resulted in an impaired vitamin A metabolism.

In rats, Emerick and Olson (1962) found that feeding nitrite but not nitrate significantly lowered liver storage of vitamin A from orally administered sources of preformed vitamin A, but not from injected sources. Nitrite in the ration also lowered vitamin A storage in the liver when carotene was fed.

Nitrate Toxicosis in Other Species

Acute Toxicosis

Saussol (1836), one of the first to record nitrate toxicosis, described poisoning of lambs by the nitrate of potash. The lambs licked the rocks and obtained sufficient amounts to cause death.

Mayo (1895) reported observations of cattle that had consumed cornstalks high in nitrate or were given large amounts of nitrate salts in a drench. This author described the darkened blood, which was due to methemoglobin, as an indication of nitrite poisoning.

Newsom et al. (1937) incriminated oat hay as the source of nitrates. Thorp (1938) and Bradley, Eppson and Beath (1940), other earlier workers on oat hay poisoning in ruminants, recommended methylene blue as an effective antidote. Besides cornstalks and oat hay they incriminated wheat, barley, and various weeds as containing sufficient nitrate to be poisonous.

In Canada, Davidson, Doughty and Bolton (1941) described signs in affected cattle as an accelerated pulse, quick breathing, trembling, staggering, and apparent blindness. The animals would cease to eat and would lie in a sternal position. Cyanosis of the tongue and sclera was followed by death with little or no struggling. Since sublethal quantities were consumed daily for a long period of time without producing toxic effects, these workers reported that nitrates had little or no cumulative effect.

Nutritional Interference Syndrome

Case (1957) discussed nitrate intoxication in livestock in Missouri. Because nitrite was considered 10 to 15 times more toxic than nitrate, animals drinking from farm ponds containing over 5 p.p.m. nitrite often suffered from nitrite intoxication. He also pointed out the dangers of nitrate accumulation by plants during droughts and heavy nitrogen fertilization. Although the cow herds were well cared for, abortion and stillborn calves were a serious problem. Blood analyses of these calves revealed methemoglobinemia. Vitamin A deficiency was evident in the dams and the hay contained 0.7% nitrate, which exceeded the safe limits.

Sippel (1960) found that a constant level of 5 p.p.m. or above of nitrite in the water could interfere with vitamin A metabolism and result in abortion, sterility, poor performance and other ills probably due to a vitamin A deficiency. He also indicated that many of the affected animals would never fully recover.

McIlwain and Schipper (1963) reported that nitrites and nitrates inhibited the conversion of carotene to vitamin A in calves. One explanation incriminated the nitrite ion as causing irritation to the mucous membranes of the gastrointestinal tract and in this manner acting as a blocking agent to carotene conversion.

Relation of Nitrites to Public Health

Used as a Meat Additive

History. Kerr et al. (1926) studied the use of nitrite in curing meats. Although nitrates were used for many years, these authors found that a small amount of nitrite produced the thermally stable meat pigment

characteristic of cured meats. In their extensive analytical studies, nitrite levels were higher in the nitrate-treated meat than in the nitrite-treated meat. From this they concluded that a small amount of nitrite produced the desired results and was no more dangerous to public health than the meat treated with nitrates. Because of this classical work, the U. S. Department of Agriculture (1960) issued regulations allowing the use of nitrite in cured products provided that,

> "The use of sodium nitrite, potassium nitrite, sodium nitrate or potassium nitrate, or combination of nitrite and nitrate, shall not result in the presence of more than 200 parts per million of nitrite in the finished product."

Toxicosis. Orgeron et al. (1957) described 10 cases of methemoglobinemia in man resulting from eating weiners and bologna high in nitrites. The clinical symptoms included nausea, vomition, profuse sweating, and an intense cyanosis of the fingertips, nose, and ears. Of 131 samples of bologna and weiners collected and analyzed, 17 contained nitrite concentrations in excess of 200 p.p.m., with the highest containing 6570 p.p.m. Although the violating plant was using a prepared curing mixture, they had recently purchased the curing ingredients separately. Instead of using the curing mixture which contained not more than 10% sodium nitrite and sodium nitrate, it appeared that pure potassium nitrite was used in preparation of the products containing high nitrite content. These authors also reported similar incidents in Florida, where 3 children became ill and 1 died after eating uncooked weiners. They stressed the employment of responsible employees on jobs where curing mixtures are formulated and emphasized the importance of state and Federal regulations concerning meat additives, such as nitrite.

Contaminated Water Sources

Water nitrite content. Although numerous reports have associated nitrate toxicosis in man and animals to drinking water, only the more pertinent references are considered in this review.

In Kansas, Metzler and Stoltenberg (1950) surveyed municipal and rural water supplies and found 19.8% of 2635 Kansas wells contained more than 10 p.p.m. NO_3-N, with the highest concentration of 486 p.p.m. By sampling 4 municipal wells at selected intervals, they found seasonal fluctuations in the nitrate levels with the highest level in winter. They also pointed out the importance of having rural, especially school, water supplies tested.

Campbell, Davis, and Myhr (1954), in Saskatchewan, reported losses of cattle on a farm where the water contained 2970 p.p.m. nitrate. This water source was an old well and was located at the edge of a barnyard where cattle wintered. These authors attributed the source of pollution to manure.

Johnston (1955) surveyed 484 Ontario wells and found 18.8% of the dug wells and 5% of the drilled wells contained NO_3-N at levels of over 10 p.p.m. This author incriminated seasonal leeching of topsoil, vegetation, fertilizers and nitrate deposits as sources of the pollution.

Faulhaber (1956), in California, reported a survey in which the same wells were sampled at selected intervals over a period of 10 years or more. Although the highest nitrate level reported was 106 p.p.m., this author believed that the nitrate content of individual wells was increasing with time. He attributed this increase to heavy fertilization of citrus crops and to sewage disposal of nearby cities and towns.

In Missouri, Case (1957) reported that many ponds contained an excess of 5 p.p.m. NO_3-N. Drought had necessitated the use of ponds for water supplies. Case (1963) further discussed the nitrate hazard of water supplies in Missouri. In one survey over 50% of the wells contained over 10 p.p.m. NO_3-N, and more than 25% contained over 100 p.p.m. He also estimated that 90% of the rural water supplies in Missouri did not meet the drinking water standards set by the U. S. Public Health Service. Although the nitrate problem in water was severe in Missouri, Iowa, and Nebraska, the problem also occurred in Canada, California, Texas, Montana, Oklahoma, Wyoming, Alabama, Florida, and Georgia. Coliform organisms from surface contamination were believed to change the nitrates to the much more toxic nitrites. This implied the increased danger of nitrite toxicosis from water in contaminated stock tanks, even though the source of water was free of coliforms.

Toxicosis. Comly (1945), in Iowa, was one of the first to diagnose cyanosis in infants due to excessive nitrates. He reported 2 cases in infants fed formula water from wells containing 140 and 90 p.p.m. NO_3-N. Examination of the wells revealed such poor construction that surface drainage contaminated the water. He also indicated that this methemoglobinemic condition occurred commonly in Iowa. In 1945, 91 wells were sampled, and the water of 56% contained over 10 p.p.m. NO_3-N. Besides the nitrate pollution, 75% of the privately owned Iowa wells were contaminated with coliform microorganisms.

Following Comly's report, Ferrant (1946) reported on 2 cases of nitrate toxicosis in infants. The wells from which the formula water was used contained 497 and 180 p.p.m. nitrates, respectively.

Faucett and Miller (1946), in Kansas, reported 3 cases of methemoglobinemia resulting from using well water of high nitrate content in infants' formulae. The first 2 cases occurred in twins, and the well water contained 70 p.p.m. NO_3-N. The third case was attributed to a well having an NO_3-N content of 300 p.p.m.

Summary

Numerous reports have clearly established the toxicity of nitrites to man and animals. The early reports dealt primarily with acute nitrite toxicosis; however, the more recent reports suggested nutritional interference syndromes as a manifestation of chronic nitrite toxicosis. The indiscriminate use of nitrites in meat food products and the accumulation of nitrites in drinking water and plant foods have caused serious illness and even death of man and animals.

OBJECTIVES

The objectives of this research were:

1. To determine the influence of prolonged nitrite ingestion upon experimental animals, as measured by (A) growth rates, (B) death losses, (C) carcass quality, (D) organ weights, (E) clinical signs, (F) hematology, and (G) gross and microscopic pathology.

2. To correlate the gross and microscopic lesions associated with nitrite ingestion with the occurrence of stomach ulcerations in pigs.

3. To determine the toxic effects of nitrite when given to anemic and nonanemic pigs.

4. To determine and record the relationship of serum nitrite to methemoglobin concentration.

5. To obtain information on the pharmacodynamics of nitrite when given to anesthetized pigs.

MATERIALS AND METHODS

Experimental Animals

The pig was used as the experimental animal because it was (1) reported as the species most susceptible to nitrite toxicosis, (2) large enough to permit ready clinical observations and collection of samples for pathologic examinations, (3) readily available at a minimum of expense, and (4) reported to have a correlation between prolonged nitrite ingestion and the gastric ulcer complex.

Facilities and General Information

For the nitrite experiments, 49 pigs were used and were maintained in Barn Number 5 on the Veterinary Research Farm, Michigan State University. In Experiment I, on chronic nitrite toxicosis, 30 pigs were used. Trials 1 and 2 were conducted using 15 pigs in each trial. The acute nitrite toxicosis studies, designated as Experiments II, III, and IV, included 4, 13, and 2 pigs, respectively. Random numbers (Dixon and Massey, 1957) were used to assign pigs to the various treatments. Nitrite stock solutions were made up as follows: Experiment I - 18.47 Gm. of potassium nitrite (KNO_2) q.s. 1000 ml. water constituted a 10,000 p.p.m. nitrite concentration, or 10 mg. of nitrite per ml. of the stock solution; and Experiments II and II - 18.47 Gm. of KNO_2 q.s. 500 ml. water constituted a 20,000 p.p.m. nitrite concentration, or 20 mg. of nitrite per ml. of the stock solution. Fresh nitrite stock solutions were made up daily.

Analytical Procedures

Hematology. Blood samples were collected from the anterior vena cava, using ethylene diaminotetraacetic acid (EDTA) or heparin as an anti-coagulant. The samples were collected at selected intervals, depending upon the experiment. Hemoglobin was determined by the cyanmethemoglobin method, packed cell volume values were determined by the micro method (capillary tube), and white blood cell counts were made using Turk's diluting fluid and a hemacytometer. At the time blood samples were collected, blood smears were made, then stained with Wright's stain and differential white blood cell counts determined.

Methemoglobin. Methemoglobin was determined by the method of Evelyn and Malloy (Hawk, Oser, and Summerson, 1954).

Serum nitrite determination. Serum nitrite-nitrogen (NO_2-N) values were determined according to the method of Diven et al. (1962a), except that concentrated mercuric chloride was used to precipitate the serum protein.

Serum vitamins A and E determinations. Vitamin A was determined according to the procedure of Sobel and Snow (1947). And vitamin E was determined with a macromodification of the Emmerie-Engel procedure employing mild saponification with ascorbic acid as an antioxidant (Hawk, Oser, and Summerson, 1954).

Liver vitamins A and E determinations. Liver vitamin A was determined according to a method of Gallup and Hoefer (1946) modified to include a saponification step with the protection of vitamin A from oxidation using

pyrogallol as an antioxidant. Vitamin E was determined by a total saponification-digestion method with a standard Emmerie-Engel reaction carried out on an aliquot of the unsaponifiable extract.

Meat nitrite determination. The nitrite concentration was determined by the standard analytical procedure of the Association of Official Agricultural Chemists (1955).

Hisopathology. Routine sections were collected, preserved in buffered 10% formalin, and were stained with hematoxylin and eosin. The histological procedures were according to the United States Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics (1957).

Specific Procedures for Each Experiment

Experiment I (Chronic Toxicosis)

Trial 1. Fifteen Yorkshire crossbred pigs, 8 weeks of age, were divided into 5 groups of 3 each and placed in separate pens. Weights of the pigs were recorded at selected intervals. Using a modified paired-feeding technique, the pigs were fed the Michigan State University swine grower ration. The amounts of feed and water were recorded daily.

In the drinking water, nitrite was given to the groups in the following amounts: Group 1, none; Group 2, 50 p.p.m.; Group 3, 100 p.p.m.; Group 4, 150 p.p.m.; and Group 5, 200 p.p.m. This was accomplished by adding 0, 20, 40, 60 and 80 ml., respectively, of the 10,000 p.p.m. stock solution q.s. 4000 ml. of water. During the experimental period, the pigs were fed and watered twice per day and were observed for signs

of toxicosis. After 10 weeks, 1 pig from each group was removed and sent to the Meats Laboratory for slaughter. The remaining 10 pigs were slaughtered at 11 weeks, when the experiment terminated. Although the animals were weighed when delivered to the Meats Laboratory at noon on the day prior to slaughter, preslaughter weights at 6 a.m. the following day were also recorded. At slaughter, blood samples were collected and the weights of the livers, kidneys, hearts, spleens, and dressed carcasses were recorded. Tissues which were placed in plastic bags, sealed and frozen at -20 F. for nitrite analyses included: ham muscle, kidney, liver, heart, spleen and lung. Liver samples were saved in a similar manner for vitamins A and E determinations. Sections from the following organs were placed in 10% buffered formalin for routine histological examination: fundic region and cardiac region of the stomach, duodenum, lung, liver, kidney, spleen, heart, superficial inguinal or supramammary lymph node, and medulla oblongata.

Trial 2. Fifteen Yorkshire crossbred pigs, 4 weeks of age, were purchased from a nearby farm. Procedures were similar to those in Trial 1, with the exceptions that higher levels of nitrite were given and methemoglobin values on the pigs were determined. In the drinking water, the nitrite was given in the following amounts: Group 1, none; Group 2, 200 p.p.m.; Group 3, 300 p.p.m.; Group 4, 400 p.p.m.; and Group 5, 500 p.p.m. This was accomplished by adding 0, 80, 120, 160, and 200 ml., respectively, of the 10,000 p.p.m. stock solution q.s. 4000 ml. of water. One pig from Group 4 (400 p.p.m.) died after 6 weeks. The remaining 14 pigs were sent to slaughter after 11 weeks.

Besides the collection of a blood sample from each pig at slaughter, blood samples were collected the fourth and seventh days of the experiment. At each time these samples were collected, the water and feed were withheld 18 hours. Then feed and nitrite water were given and blood samples collected 2 hours later. In addition to the routine hematology, methemoglobin values were determined.

Experiment II (Acute Toxicosis)

Four Yorkshire crossbred pigs were used, Pigs Nos. 1 and 2 were purchased from a nearby farm, and Nos. 3 and 4 were raised at the Veterinary Research Farm. Each day, the pigs were weighed and an initial blood sample was collected. After oral dosing with the calculated amount of nitrite, blood samples were collected each hour for 4 hours for routine hematology and methemoglobin analyses. The nitrite was administered by using a small plastic stomach tube and a 30 ml. glass syringe. After administration of the nitrite, 20 ml. of water were placed in the syringe and injected through the plastic tube into the stomach to assure that none of the nitrite solution remained in the tube. For 4 hours after dosing, the pigs were observed and the clinical signs recorded. At necropsy the tissues preserved for histological examination were the same as those listed for Experiment I.

Experiment III (Anemia)

Thirteen Yorkshire crossbred baby pigs were born November 25, 1963, and left with the dam throughout the experimental period. At 1 week of age, all were weighed and ear notched. At this time 7 were given 2 ml.

of an iron preparation (Armidextran, Armour & Co.) intramuscularly. At 14 and 21 days of age, weights were again recorded. The group of pigs given the iron injections are designated as iron-treated and the other group as nontreated.

Initial blood samples plus samples at selected intervals were collected. In addition to routine hematology and methemoglobin analyses, serum NO_2-N concentrations were also determined. At death, or when euthanatized, each pig was necropsied and gross changes were recorded. Sections preserved for histologic examination from Pigs 5, 6, 30, and 50 included heart, spleen, kidney, and liver. Spleen and liver weights were recorded for all pigs.

Experiment IV (Pharmacodynamics)

Two Yorkshire crossbred pigs, each weighing 45 pounds, were purchased from a nearby farm. Each pig was given 50 mg. Sparine (Wyeth) intramuscularly, followed by an intravenous injection of 6% sodium pentobarbital to produce surgical anesthesia.

In the first pig, the anterior vena cava, both carotid arteries, and femoral artery were cannulated. A tracheal tube was inserted into the trachea by surgical means. A Grass Polygraph 4-channel recorder (Model 5, Grass Instrument Company, Quincy, Massachusetts) recorded the blood pressure values, EKG, and respirations. For the blood pressure recordings, Channel 1 and 5P1 amplifier with a Statham high pressure transducer was used. Using Lead 2, the EKG was recorded by Channel 2 and 5P4 amplifier. Respirations were recorded by Channel 3 and a 5P1 amplifier with a PT5A low pressure air transducer. The nitrite (30 mg./lb. body weight) was dissolved in 10 ml. of water and administered directly

into the stomach which was exposed by laparotomy. After collecting an initial blood sample, subsequent samples were collected at 30-minute intervals from the anterior vena cava, carotid artery, saphenous vein, and femoral artery.

In the second pig, the carotid artery was cannulated and the tracheal tube inserted. A laparotomy exposed the stomach and the abdominal blood vessels. Again, the same recording devices were used. Blood samples were drawn initially and at 30-minute intervals from the posterior vena cava at the diaphragm and posterior to the kidneys, the renal vein, and the aorta posterior to the kidneys. The nitrite (30 mg./lb body weight) was injected into the stomach.

For collection of the blood samples, heparin was used as an anticoagulant. Methemoglobin and serum NO_2-N values were determined. Routine hematology was determined at each bleeding interval on the blood sample from the carotid artery of the first pig and from the aorta of the second pig. At death, both pigs were necropsied and observed for gross changes.

RESULTS

Experiment I

Growth

The data on the growth rate of pigs given various levels of nitrite in their drinking water are summarized in TABLE 1-1. In each of the 2 trials, no major differences are noted for the various treatments. All pigs gained well during the experimental period. Figure 1-1 is presented to illustrate the size and general condition of a pig given 200 p.p.m. nitrite in the drinking water for 10 weeks. The average daily feed, water, and nitrite consumption of pigs given the highest level of nitrite in each trial is presented in TABLE 1-2.

Analyses

Slaughter data. The data collected at slaughter are given in TABLE 1-3. No major differences are noted in the dressing percentage or in the liver, kidney, heart or spleen weights. Gross examination of the carcasses and primal cuts failed to reveal any significant alterations in the color or other quality characteristics. Figure 1-2 illustrates the carcasses of pigs given various amounts of nitrite in their drinking water for 10 weeks.

Hematology. The hematological data from the pigs of Trials 1 and 2 are given in TABLE 1-5. No major differences are noted in the hemoglobin (Hb.) or packed cell volume (PCV) values or the white blood cell (WBC) values at slaughter. The WBC values of blood collected on days

TABLE 1-1.--Experiment I. Average Growth Rate of Pigs Given Selected Levels of Nitrite in the Drinking Water.

Group No.	No. of Pigs	Treatment (p.p.m. NO_2)	Average Initial Wt.(lb.)	Average Final Wt. (lb.)	Average Daily Gain (lb.)
		Trial 1 (77 days)			
1	3	0	47	186	1.87
2	3	50	49	189	1.88
3	3	100	46	184	1.84
4	3	150	47	182	1.81
5	3	200	47	191	1.90
		Trial 2 (77 days)			
1	3	0	15	107	1.20
2	3	200	17	98	1.05
3	3	300	17	105	1.14
4	2	400	14	108	1.20
5	3	500	15	103	1.16

TABLE 1-2.--Experiment I. Average Daily Feed, Water, and Nitrite Consumption for Pig Given Nitrite in the Drinking Water in Group 5 of Each Trial.

Week of Experiment	Feed (lb./day)	Water (gal./day)	Intake of NO_2 (mg.)	
			Total/day	Per lb.
		Trial 1, Group 5 (200 p.p.m.)		
1	3.0	0.62	496	9.1
2	4.3	0.82	688	10.0
3	4.4	1.14	912	11.1
4	4.6	1.28	1024	10.8
5	4.7	1.33	1064	10.1
6	5.4	1.33	---	---
7	6.0	1.33	---	---
8	7.6	1.71	1366	10.4
9	8.0	1.71	---	---
10	9.0	1.71	1366	8.2
11	9.0	1.71	1366	7.5
		Trial 2, Group 5 (500 p.p.m.)		
1	0.7	0.29	580	28.3
2	1.4	0.33	660	26.1
3	2.0	0.48	960	30.2
4	2.4	0.62	1240	30.4
5	2.7	0.76	---	---
6	2.7	0.86	1720	30.7
7	2.7	0.90	---	---
8	3.0	1.00	---	---
9	3.8	1.00	---	---
10	4.0	1.10	---	---
11	5.0	1.20	2400	28.2

4 and 7 from pigs in Trial 2, given nitrite, indicate a leukocytosis with an absolute neutrophilia. Methemoglobin (MetHb.) values up to 0.5 Gm./100 ml. of blood are noted in Groups 4 and 5 during Trial 2.

Serum and liver vitamins A and E determinations. Liver and serum vitamins A and E values are presented in TABLE 1-4. Differences are noted between treatments but could not be correlated with nitrite consumption.

Nitrite residue determinations. No nitrite residues were present in the lungs, kidneys, spleen, liver, or heart from the pigs in Trial 1.

Signs

The pigs given 200 p.p.m. nitrite and above were occasionally cyanotic, dyspneic, listless, and lethargic within 30 minutes after eating and drinking. These signs usually subsided within 2 hours and the pigs appeared normal again. One pig in the 400 p.p.m. group died the 8th week of the experiment following signs of acute nitrite toxicosis.

Gross Lesions

At necropsy, the pig that died during the experiment had a brownish discoloration of the blood, edema of the lungs, and congestion of the visceral and peripheral blood vessels. At slaughter, some of the remaining pigs revealed a keratinization of the stomach, ulcers in the cardial region of the stomach, and scarring of the liver. These lesions were noted in the control pigs as well as in the nitrite-treated pigs.

Microscopic Lesions

No significant pathological changes were found, except in sections from the stomachs and livers of some of the pigs. The lesions in the

Fig. 1-1.--A pig given 200 p.p.m. nitrite in the drinking water for 10 weeks.

Fig. 1-2.--Carcasses of pigs given nitrite in the drinking water for 10 weeks. Dosage levels from left to right: 200, 150, 100, 50, and 0 p.p.m.

TABLE 1-3.--Experiment I. Slaughter Data (Averages) Collected From Pigs Given Selected Levels of Nitrite in the Drinking Water

Group No.	No. of Pigs	Treatment (p.p.m. NO_2)	Live Wt. (lb.)	Dressing %	Organ Weights (Gm.)			
					Liver	Kidneys	Heart	Spleen
			Trial 1					
1	3	0	169	74	1511	313	273	129
2	3	50	171	75	1528	307	267	112
3	3	100	166	74	1457	328	250	110
4	3	150	162	75	1415	272	250	105
5	3	200	172	75	1568	333	273	117
			Trial 2					
1	3	0	102	83	1004	195	192	82
2	3	200	92	83	1016	170	183	59
3	3	300	99	84	926	190	169	76
4	2	400	102	83	1035	187	220	92
5	3	500	97	84	983	157	173	73

TABLE 1-4.--Experiment I. Average Vitamin A and Vitamin E Values of Liver (mcg./Gm.) and Serum (mcg./100 ml.) from Pigs Given Selected Levels of Nitrite in the Drinking Water.

Group No.	No. of Pigs	Treatment (p.p.m. NO_2)	Vitamin A		Vitamin E	
			Liver	Serum	Liver	Serum
		Trial 1				
1	3	0	186	43	10	116
2	3	50	242	63	11	380
3	3	100	221	41	12	26
4	3	150	196	41	9	386
5	3	200	253	44	11	71
		Trial 2				
1	3	0	69	41	5	245
2	3	200	64	58	4	193
3	3	300	93	62	6	245
4	3	400	58	52	5	241
5	3	500	74	39	5	225

TABLE 1-5.--Experiment I. Modified Hemogram of Swine Given Selected Levels of Nitrite in Drinking Water.

Group No.	No. of Pigs	Treatment (p.p.m. NO_2)	Hb. (Gm./ 100 ml.)	MetHb. Gm./ 100 ml.)	PCV %	WBC (Cells/ cmm.)	WBC Differential Counts (%) PMN	Stab	Lymph	Mono	Baso	Eosin
					Trial 1							
					At Slaughter							
1	2	0	13.3	---	41.5	20,050	17	1	80	1	0	2
2	3	50	11.9	---	40.7	25,000	26	4	64	3	1	3
3	3	100	13.9	---	40.7	21,600	16	3	75	3	0	3
4	2	150	11.3	---	34.5	19,850	24	5	67	1	0	5
5	2	200	12.5	---	42.0	18,350	16	5	76	1	1	3
					Trial 2							
					Day 4*							
1	3	0	9.0	0.00	29.7	22,600	42	3	53	1	0	0
2	3	200	8.5	0.00	28.7	31,570	33	3	63	0	0	1
3	3	300	8.6	0.00	28.3	25,100	31	4	65	0	0	1
4	3	400	9.3	0.16	30.0	38,300	49	4	45	1	0	2
5	3	500	9.0	0.49	29.0	27,470	53	4	42	1	0	1
					Day 7*							
1	3	0	9.0	0.00	30.3	22,300	33	1	67	0	0	0
2	3	200	8.3	0.00	26.7	28,100	35	6	57	0	0	2
3	3	300	9.0	0.00	28.7	23,470	39	3	57	0	0	1
4	3	400	9.0	0.16	30.0	32,730	52	4	44	0	0	0
5	3	500	9.5	0.49	31.7	30,200	51	3	45	1	0	1
					At Slaughter							
1	2	0	14.5	0.00	42.5	20,100	18	1	77	2	0	3
2	2	200	13.3	0.00	39.5	21,050	26	2	73	0	0	0
3	3	300	14.4	0.00	41.7	17,870	22	3	71	1	0	3
4	2	400	14.8	0.00	43.0	21,550	28	1	69	2	0	1
5	2	500	14.2	0.10	42.5	17,500	21	1	77	1	0	1

*Feed and water withheld 18 hours, then given free choice and blood samples collected 2½ hours later.

stomach were characterized by a parakeratotic proliferation of the squamous epithelium in the cardiac region. The ulcers in the same region were characterized by a break in the continuity of the epithelium, lymphocytic and neutrophilic infiltration, and the presence of erythrocytes on the ulcerated surface. The lesions in the liver were characterized as a chronic eosinophilic hepatitis and consisted of a perilobular infiltration of eosinophils with an accompanying proliferation of fibroblasts and bile duct epithelium.

Experiment II

Analyses

The blood values from pigs given the initial dose of nitrite by stomach tube are given in TABLE 1-6. Except for the animal that died, the methemoglobin values reached a peak in 1 to 2 hours after dosing. At death, all the hemoglobin was oxidized to methemoglobin in Pig 1. The average total WBC counts increased to the highest level in 1 to 3 hours after administration of nitrite. During the first 4 hours after dosing, the neutrophil count increased and the lymphocyte and eosinophil counts decreased. The blood counts of Pigs 3 and 4 revealed 20 and 22 eosinophils per 100 WBC prior to dosing with nitrite but 4 hours later only 1 eosinophil per 100 WBC was found in 1 pig and no eosinophils in the other. This observation is of importance to the interpretation of the microscopic findings. The blood values given in TABLE 1-6 are essentially the same as those obtained when Pigs 2, 3, and 4 were given the same dosage of nitrite on 3 additional successive days.

TABLE 1-6.--Experiment II. Modified Hemogram from Representative Swine Given Selected Levels of Nitrite by Stomach Tube

Pig No.	Treatment (mg. NO_2/ lb.)	Hours After Dosing	Hb. (Gm./ 100 ml.)	MetHb. (Gm./ 100 ml.)	PCV (%)	WBC (Cells/ cmm.)	WBC Differential Counts (%)					
							PMN	Stab	Lymph	Mono	Baso	Eosin
2	20	0	6.8	0.0	23	14,300	33	1	66	0	0	0
		1	6.2	2.7	22	16,200	48	10	41	1	0	0
		2	6.8	2.0	23	23,000	41	25	34	0	0	0
		3	7.0	1.2	23	24,300	63	22	14	1	0	0
		4	6.8	0.5	21	19,900	60	32	8	0	0	0
		7	6.2	0.0	21	20,500	64	13	23	0	0	0
3	25	0	13.3	0.0	43	15,300	27	2	51	0	0	20
		1	11.3	4.7	41	17,200	47	2	38	0	0	13
		2	13.3	5.6	41	16,600	50	10	35	0	0	5
		3	12.3	3.0	39	16,300	56	5	38	0	0	1
		4	12.0	1.7	38	14,400	47	22	30	0	0	1
		7	13.0	0.2	39	14,400	54	4	37	2	0	3
4	30	0	13.0	0.2	42	14,000	22	11	44	1	0	22
		1	12.0	4.7	41	19,500	22	10	45	0	0	23
		2	11.6	4.4	40	20,200	52	13	30	0	1	4
		3	12.6	3.5	38	11,700	57	12	29	0	0	2
		4	12.6	2.2	37	13,700	51	6	43	0	0	0
		7	13.0	1.0	38	13,900	56	2	41	0	0	1
1	30	0	5.9	0.0	22	10,000	34	0	65	0	0	1
		1	4.8	3.3	20	18,800	53	4	42	1	0	0
		2	5.1	3.8	20	16,000	40	3	57	0	0	0
		3*	4.4	4.4	18	8,200	60	10	30	0	0	0

*Died

Signs

The signs listed in TABLE 1-7 are typical of all the pigs dosed with nitrite by stomach tube; only the intensity and termination varied. Pigs 1 and 4 died with signs of acute nitrite toxicosis. Pigs 2 and 3 were euthanatized at the end of the experiment. Death usually occurrred within 4 hours, and by 6 hours the pigs generally appeared normal.

Gross Lesions

At necropsy, Pigs 1 and 4 had extreme cyanosis and brownish discoloration of the blood and tissues. The stomach of Pig 4 had hemorrhages and ulcerations in the fundic region (Fig. 1-3). The livers of Pigs 3 and 4 had numerous whitish foci 1 cm. in diameter and extended into the parenchyma. Fig. 1-4 illustrates these foci and also the evidence of methemoglobinemia.

Microscopic Lesions

Histopathologic examination of the lungs of the pigs dying of acute nitrite toxicosis had areas of emphysema, edema, and congestion (Fig. 1-5). The livers of Pigs 3 and 4 contained numerous areas of perilobular eosinophilic infiltration and fibroblastic and bile duct proliferation (Fig. 1-6). Numerous eosinophils and edema were present in the fundic portion of the stomachs of Pigs 3 and 4 (Figs. 1-7 and 1-8). Hemorrhages and ulcerations were also present in the stomach of Pig 4.

TABLE 1-7.--Experiment II. Characteristic Signs of Pigs with Acute Nitrite Toxicosis. Pig 1 Given 30 mg. of Nitrite/lb. Body Weight by Stomach Tube.

Minutes After Dosing	Signs
0	Normal
15	Salivation
30	Defecation, stretching
40	Urination
45	Ataxia, restless, skin blanched
60	Vomition, dyspnea, abdominal pain
70	Urination, abdomen tucked up, quivering
80	Vomition, salivation
90	Marked ataxia, weakness
100	Urination, buccal mucosa cyanotic
120	Marked dyspnea, side paddling
140	Severe convulsions
160	Severe dyspnea
175	Gasping
180	Death

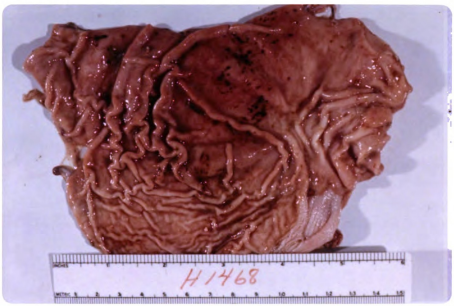

Fig. 1-3.--Stomach of Pig 4, given 30 mg. nitrite/lb. body weight by stomach tube for 4 consecutive days. Note hemorrhages and ulceration of the fundic region.

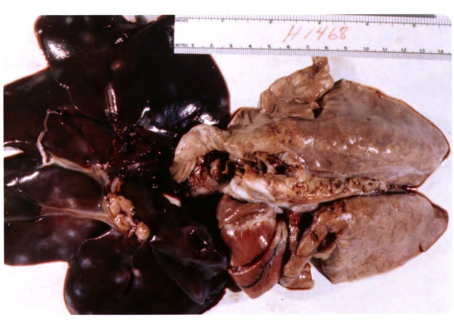

Fig. 1-4.--Liver, heart, and lungs of Pig 4, which died after being given 30 mg. nitrite/lb. body weight by stomach tube for 4 consecutive days. Note the brownish color of blood and lungs.

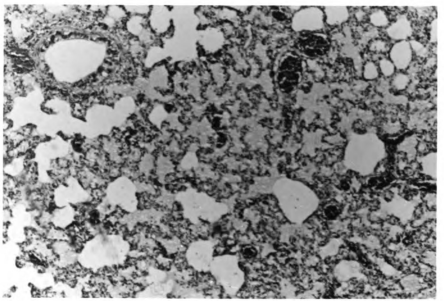

Fig. 1-5.--Section from lung in Fig. 1-4. Note alveolar edema, emphysema and the congestion of the blood vessels. H & E stain; x 75.

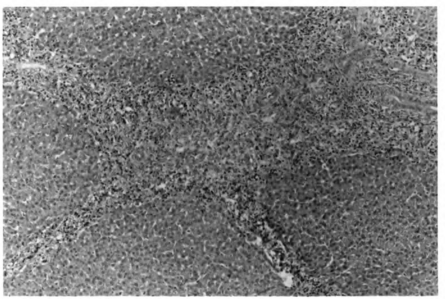

Fig. 1-6.--Section from liver in Fig. 1-4. Note the perilobular infiltration of eosinophils and the fibroblastic and bile duct proliferation. H & E stain; x 75.

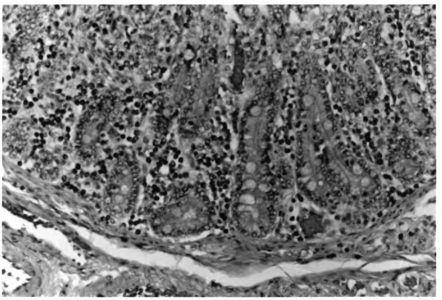

Fig. 1-7.--Eosinophilic infiltration of fundic region of stomach shown in Fig. 1-3. H & E stain; x 187.

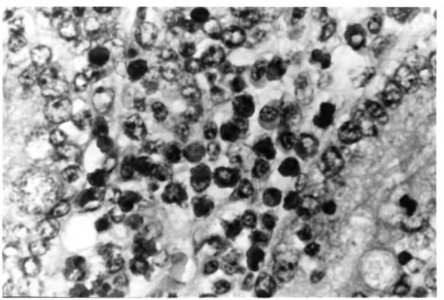

Fig. 1-8.--Higher power of Fig. 1-7. Note the eosinophils and the edema of the fundic region. H & E stain; x 750.

Experiment III

Growth

The data on weight gains for the iron-treated and nontreated pigs are given in TABLE 1-8. The pigs given iron intramuscularly gained 2 pounds per pig more than the nontreated group during the 2-week period.

Analyses

Organ weights. Spleens of the iron-treated pigs were larger than the spleens of the nontreated pigs per unit body weight. There were no major differences in the average liver weights of pigs from the 2 groups (TABLE 1-8).

Hematology. Hemoglobin, methemoglobin, PCV and serum nitrite-nitrogen (NO_2-N) values and the total and differential WBC counts are given as averages for each group in TABLE 1-9. Prior to dosing with nitrite, the average hemoglobin value for the iron-treated pigs was 10.8 Gm. and for the nontreated pigs 4.0 Gm./100 ml. of blood. The PCV values are in direct proportion to the hemoglobin values, with an average of 37.0% for the iron-treated and 15.5% for the nontreated pigs. Methemoglobin and serum NO_2-N values were not detected prior to dosing with nitrite. The average total WBC counts are slightly lower for the nontreated group but the differential WBC counts for the 2 groups indicate no major differences. The nucleated red blood cells (NRBC) per 100 WBC counted, however, averaged 14 for the nontreated group and 3 for the iron-treated group.

The methemoglobin values parallelled the hemoglobin values and were both much higher in the iron-treated group than in the nontreated group.

TABLE 1-3.--Experiment III. Average Growth Rate and Organ Weights of Iron-Treated (Group 1) and Nontreated (Group 2) Pigs.

Group Number	No. of Pigs	Days of Age			At Necropsy	
		7 (lb.)	14 (lb.)	21 (lb.)	Liver (Gm.)	Spleen (Gm.)
1	7	5.5	9.7	12.3	199	15
2	6	5.8	8.9	10.5	176	9

TABLE 1-9.--Experiment III. Summary of Modified Hemograms (Avg.) from Iron-Treated (Group 1) and Non-Treated (Group 2) Pigs Given Selected Levels of Nitrite by Stomach Tube.

Group No.	No. of Pigs	Minutes After Dosing	Hb. (Gm./ 100 ml.)	MetHb. (Gm./ 100 ml.)	PCV (%)	Serum NO_2-N (mcg./100 ml.)	WBC Cells/ cmm.)	Nucleated RBC	WBC Differential Counts (%)					
									PMN	Stab	Lymph	Mono	Baso	Eosin
Before Dosing with Nitrite														
1	7	0	10.8	0.0	37	0	8,440	3	27	1	70	1	0	1
2	6	0	4.0	0.0	15	0	7,870	14	29	0	69	1	0	0
25 mg. Nitrite/lb.														
1	2	150	10.3	4.5	34	13	12,400	4	60	1	39	0	0	0
2	2	150	3.5	1.5	13	80	7,950	10	43	2	55	0	0	0
30 mg. Nitrite/lb.														
1	3	150	10.1	6.3	35	0	12,020	3	56	0	43	1	0	0
2	3	150	3.9	1.8	14	275	12,160	8	55	3	45	0	0	0
32 mg. Nitrite/lb.														
1	2	15	9.3	0.9	30	128	16,250	3	37	1	60	2	0	0
2	3	15	3.6	0.5	12	782	7,250	11	43	1	55	0	0	0
1	2	30	9.0	2.2	29	262	15,250	5	41	0	59	0	0	0
2	3	30	3.4	1.0	13	1062	7,530	16	37	1	62	0	0	0
1	3	60	9.0	4.3	29	395	19,100	3	53	2	44	1	0	0
2	4	60	3.4	1.3	14	1392	8,600	17	28	2	71	0	0	0
1	4	90	9.2	6.6	30	205	18,350	4	51	1	47	2	0	0
2	3	90	3.1	1.9	11	758	7,570	22	23	2	73	1	0	0
1	4	150	10.0	7.1	33	184	17,800	4	47	3	47	1	0	0
2	3	150	3.7	1.8	14	498	11,030	20	45	3	51	1	0	0
1	1	240	9.3	2.7	29	73	17,900	0	71	5	22	2	0	0
2	1	240	4.3	1.2	16	285	7,500	7	58	2	40	0	0	0

In general, methemoglobin values were highest at 2 hours after dosing in the pigs that survived. In pigs that died, values were the highest at the time of death. In contrast to the methemoglobin values, the serum NO_2-N values were highest at 1 hour after dosing with nitrite. No correlation was noted between the serum NO_2-N values and methemoglobin values in a specific pig at a given time after dosing with nitrite. In general, the total WBC counts increased after nitrite treatment in the iron-treated pigs much more than in the nontreated pigs. An absolute neutrophilia was also noted.

Signs

The signs observed were similar to those described in TABLE 1-7 (Experiment II); only the intensity varied. Fig. 1-9 illustrates some of the characteristic signs of acute nitrite toxicosis. Although death followed convulsions in most of the pigs, a few fully recovered spontaneously without treatment within 2 to 3 hours. Blood samples from pigs that recovered contained low levels or no methemoglobin at 7 hours after nitrite administration. The LD_{50} of nitrite, dosed by stomach tube to swine, was 30 to 32 mg./lb. body weight. No major differences were noted in the LD_{50} for the iron-treated or nontreated groups.

Gross Lesions

Fig. 1-10 illustrates the paleness of the skin, nose and mucous membranes of the nontreated pig in contrast to the pink color of the iron-treated pig at necropsy. The viscera of the nontreated pig were pale, and the blood was watery and thin (Fig. 1-11). The pigs dosed with nitrite had brownish discoloration of the blood and tissues, and

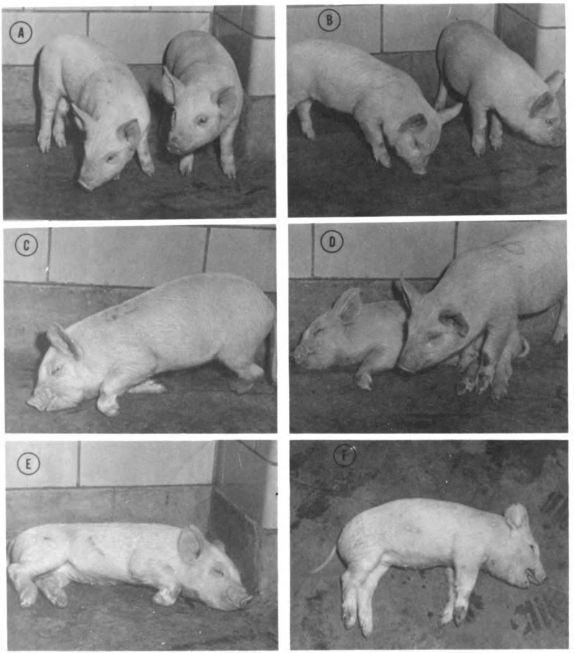

Fig. 1-9.--Experiment III. Signs of pigs given 32 mg. nitrite/lb. body weight by stomach tube. (A) normal, (B) ataxia, (C) weakness and ataxia, (D) unable to rise, (E) comatose, and (F) gasping shortly before death.

Fig. 1-10.--Iron-treated and nontreated pigs. Note the paleness of nose and ears of the nontreated pig on the left.

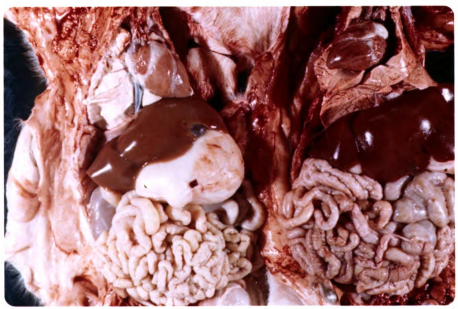

Fig. 1-11.--Viscera of iron-treated and nontreated pigs shown in Fig. 1-10. Note the paleness of the visceral organs and blood of the nontreated pig on the left.

the lungs appeared blanched. Only the intensity of the brownish discoloration varied with the iron-treated and nontreated groups (Figs. 1-12 and 1-13).

Microscopic Lesions

On histopathologic examination of the lungs of pigs with acute nitrite toxicosis, congestion and edema were present, and in other organs and tissues, only congestion was present.

Experiment IV

Analyses

Some of the pharmacodynamic effects of 30 mg. nitrite/lb. body weight injected into the stomach of anesthetized pigs were recorded until the time of death. Information on blood pressure values and heart and respiratory rates is summarized in TABLE 1-10. The systolic and diastolic blood pressure values decreased rapidly after injection of nitrite into the stomach. Since the systolic pressure decreased more than the diastolic pressure, the pulse pressure also decreased. The heart and respiratory rates, in general, increased during the experimental period, except near death, when both decreased rapidly. TABLE 1-10 also illustrates that death is apparently due to respiratory failure rather than to cardiac failure. Electrocardiographic tracings were essentially the same before and after nitrite administration.

The hemoglobin, methemoglobin and serum NO_2-N values for various locations are given in TABLE 1-11. The methemoglobin values increased during the experimental period, and no major differences were noted in the values determined from arterial or venous blood. The serum NO_2-N

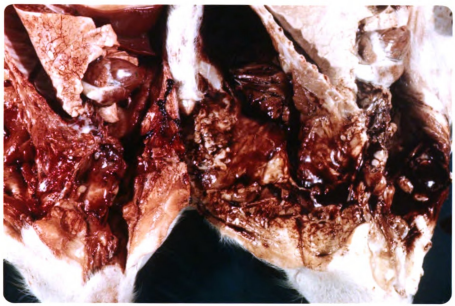

Fig. 1-12.--Thoracic and neck area of 2 iron-treated pigs. The pig on the right died after given 32 mg. nitrite/lb. body weight by stomach tube. Note the brownish-colored blood and blanched heart and lungs in the pig on the right, in contrast to the control pig on the left.

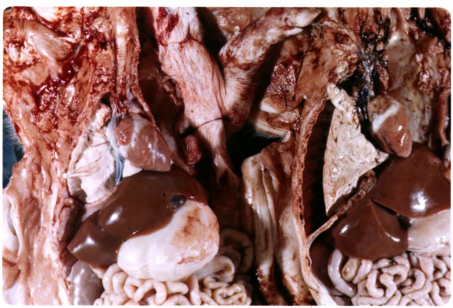

Fig. 1-13.--Visceral and neck region of 2 nontreated pigs. The pig on the right died after given 32 mg. nitrite/lb. body weight by stomach tube. Note the brownish watery blood and blanched lungs in the pig on the right, in contrast to the control pig on the left.

TABLE 1-10.--Experiment IV. Blood Pressure Values and Heart and Respiratory Rates of an Anesthetized Pig Given 30 mg. Nitrite/lb. Body Weight by Injection into the Stomach.

Minutes After Injection	Blood Pressure (mm. Hg) Systolic	Diastolic	Pulse	Heart Rate (per Minute)	Respirations (per Minute)
0	150	120	30	134	28
3	135	105	30	150	24
5	105	80	25	180	20
10	75	50	25	174	22
20	75	50	25	156	26
30	80	55	25	150	30
45	80	60	20	180	28
60	80	60	20	174	26
90	95	75	20	198	36
120	85	65	20	234	36
130	75	55	20	240	30
144	60	45	15	234	24
145	60	45	15	234	0
150	50	35	15	180	0
151	0	0	0	0	0

TABLE 1-11.--Experiment IV. Hb., Meth., and Serum NO_2-N Values of Blood Collected in Different Locations from Anesthetized Pigs Given 30 mg. Nitrite/lb. Body Weight by Injection into the Stomach

Pig No.	Bleeding Site	Minutes After Injection 0	30	60	90	120	150
	Hemoglobin (Gm./100 ml.)						
1	Carotid artery	9.6	10.0	9.9	11.0	12.0	12.0
2	Aorta	8.8	10.3	10.5	---	---	---
	Methemoglobin (Gm./100 ml.)						
1	Carotid artery	0.0	2.0	2.9	3.9	4.7	5.1
	Femoral artery	0.0	1.7	2.9	4.4	4.4	5.4
	Anterior vena cava	0.0	2.0	3.2	4.2	4.4	5.4
	Saphenous vein	0.0	1.5	3.4	4.4	4.9	5.3
2	Aorta	0.0	0.7	1.5	---	---	---
	Posterior vena cava	0.0	0.7	1.7	---	---	---
	Renal vein	0.0	0.7	1.7	---	---	---
	Serum NO_2-N (mcg./100 ml.)						
1	Carotid artery	0	390	130	330	150	150
	Femoral artery	0	450	200	390	150	170
	Anterior vena cava	0	310	130	270	90	140
	Saphenous vein	0	200	100	70	90	130
2	Aorta	0	170	150	---	---	---
	Posterior vena cava	0	70	75	---	---	---
	Renal vein	0	25	35	---	---	---

values were highest 30 minutes after administration of nitrite and were higher in the arterial blood than in the venous blood. The blood from the renal vein contained considerably less serum NO_2-N than either the aorta or posterior vena cava. Further, since NO_2-N was present in the urine, it was concluded that some of the nitrite is eliminated in the urine.

Gross Lesions

Marked cyanosis, congestion of the visceral and peripheral blood vessels, and brownish discoloration of the blood and tissues were evident at necropsy.

DISCUSSION

Experiment I

Prolonged nitrite ingestion had no significant effect on growth, dressing percentages or organ weights; on the white blood cell, hemoglobin or packed cell volume values; or on the serum and liver vitamins A and E values. No nitrite residues were present in the lungs, kidneys, livers, spleens, or hearts. Pigs given higher levels (300, 400, or 500 p.p.m.) of nitrite in the water were occasionally listless, cyanotic, and lethargic shortly after eating and drinking. Case (1963) reported nutritional interference syndromes in pigs given water containing 50 p.p.m. nitrate-nitrogen (NO_3-N) and also that nitrite was at least 10 times more toxic than nitrate. By conversion of the nitrite to NO_3-N and dividing by 10, 16 to 17 p.p.m. nitrite should have caused the interference syndromes in this study. Some of the pigs in this experiment were given 30 times this calculated dose, and there was no evidence of chronic toxicosis. The pig that died while consuming 400 p.p.m. nitrite in the water had evidence of methemoglobinemia but evidence of chronic toxicosis either grossly or microscopically was not present. These facts indicate that the chronic nitrate-nitrite toxicosis under farm conditions was not reproduced under these experimental conditions. This might suggest that toxic factors other than nitrite may partially be responsible for the toxicosis observed by other workers.

The gastric ulcerations and other subacute to chronic lesions believed due to nitrite ingestion by Bush and Matteson (1963) were not observed. The gastric ulcerations and chronic eosinophilic hepatitis

observed at necropsy of these experimental animals were similar to those seen routinely at slaughter. In addition, no increase in the incidence of these conditions was noted in the pigs given nitrite in their drinking water. In regard to the possibility of a cumulative effect by nitrite, the pigs given 500 p.p.m. nitrite in their drinking water consumed daily nearly an LD_{50} amount of nitrite if it had been given in a single dose. This lack of any accumulative effects of daily consumption of nitrite supported the research of Laing and McIntosh (1947).

The liver vitamins A and E values from the pigs in this experiment were not significantly different from the controls. This information is in contrast to the reports of Hvidsten (1955) and Bjornson et al. (1961), who suggested that nitrite interfered with vitamins A and E metabolism. The liver vitamins A and E determinations revealed more consistent values than the serum determinations.

Experiments II, III, and IV

General Observations

These experiments on acute nitrite toxicosis suggested the LD_{50} for swine was approximately 30 to 32 mg. nitrite/lb. body weight. This confirmed the investigations of Stormorken (1953). It was particularly significant that no differences were noted in the LD_{50} for the non-treated (anemic) and iron-treated (nonanemic) pigs of Experiment III.

While the signs of acute nitrite toxicosis included those of anoxia, the data in Experiment IV suggested the ultimate cause of death as respiratory rather than cardiac failure. The gross pathologic observations included brownish discoloration of the blood and tissues and congestion of the visceral and peripheral blood vessels.

Hematology

Several hematological findings associated with nitrite dosing by stomach tube merit discussion. First, the total white blood cell counts increased during the first hour but then decreased in 4 to 7 hours to near the predosing value. Secondly, the number of circulating neutrophils increased during the first 4 hours after dosing. The total white cell counts decreased to nearly the predosing values in 4 to 7 hours, which indicated an absolute neutrophilia. Thirdly, the number of circulating lymphocytes decreased during the first 4 to 7 hours after nitrite dosing and, therefore, an absolute lymphopenia was noted. Lastly, 2 pigs had high eosinophil counts prior to dosing with nitrite, but 4 hours later the eosinophil counts were near zero. Subsequent histopathologic study of the gastric mucosa indicated the infiltration of numerous eosinophils. These histopathologic findings were not present in the pigs with low eosinophil counts prior to dosing with nitrite. Therefore, further investigations into the role of eosinophils in toxicoses such as nitrite are suggested.

Methemoglobin

In all the pigs that survived, the highest methemoglobin levels were found 1 to 2 hours after dosing with nitrite. In the pigs that died of acute nitrite toxicosis, the highest methemoglobin values were found at death. Pigs with low hemoglobin values had lower methemoglobin values after the same dosages than pigs with high hemoglobin values. However, a direct proportion of methemoglobin to hemoglobin was noted in each group of pigs given the same per pound dosages of nitrite. This would indicate that the natural body protective mechanisms, as described

by Huennekens et al. (1957), may have reduced the methemoglobin to the functional hemoglobin. These latter observations were confirmed in this study as there appeared to be a point of maximum methemoglobin formation unless the natural protective mechanisms were overwhelmed. If overwhelmed, the methemoglobin formation continued and death occurred. This body protective mechanism appeared to control the proportion of methemoglobin to hemoglobin, regardless of the amount of hemoglobin.

Serum Nitrite-Nitrogen

The results of the serum nitrite-nitrogen (NO_2-N) determinations indicated that the highest values occurred about 30 minutes following dosing with nitrite. As expected, these high serum values occurred prior to the highest methemoglobin values. However, the serum NO_2-N values were not in proportion to the amount of the methemoglobin that eventually formed. The iron-treated pigs with an average hemoglobin value of 10.8 Gm./100 ml. had lower serum NO_2-N values than nontreated pigs with an average hemoglobin value of 4.0. Methemoglobin values were highest in the iron-treated pigs. From this, it is concluded that the serum NO_2-N values are not indicative of the amount or proportion of methemoglobin that is eventually formed. These findings indicate the need for further investigational work with nitrite reduction products, especially hydroxylamine and ammonia.

Pharmacodynamics

The pharmacodynamic effects of nitrite upon anesthetized pigs included decreased diastolic, systolic, and pulse pressures and increased heart and respiratory rates. No major differences were noted in the

electrocardiograms. The differences in serum NO_2-N values in the renal vein from values in either the aorta or posterior vena cava and the presence of NO_2-N in the urine indicated that some of the nitrite was excreted unchanged by the kidneys. This experiment indicated the possibilities of using pigs under anesthesia for future work in studying acute toxicoses.

SUMMARY

Four experiments were conducted using a total of 49 pigs to evaluate and study nitrite toxicosis in swine. Thirty pigs in 2 different experiments were given up to 500 p.p.m. nitrite in their drinking water for 11 weeks. No major differences due to nitrite were observed in the growth rates, dressing percentages, organ weights, or white blood cell, hemoglobin or packed cell volume values. In addition, serum and liver vitamins A and E values were not influenced by nitrite. No nitrite residues were found in the muscle tissue or vital organs. Higher levels of nitrite occasionally caused listlessness, slight cyanosis, and lethargy in pigs shortly after eating and drinking. No lesions attributable to prolonged nitrite ingestion were found on gross and histopathologic examinations. The incidence of gastric ulcerations was not increased by the prolonged nitrite ingestion.

Nineteen pigs in 3 different experiments were used to study acute nitrite toxicosis. The LD_{50} of nitrite orally for pigs was calculated as 30 to 32 mg./lb. body weight. Anemia had no effect on the LD_{50}. Signs of acute nitrite toxicosis were primarily those of anoxia and death was apparently due to respiratory failure. Hematological studies during the 4 hours following nitrite dosing indicated a transitory leukocytosis and an absolute neutrophilia, lymphopenia and eosinopenia. Methemoglobin and serum nitrite-nitrogen (NO_2-N) values were not related to different hemoglobin values in pigs. Decreased diastolic, systolic, and pulse pressures and increased heart and respiratory rates were

noted after injection of nitrite into the stomach. Gross lesions in acute toxicosis included brownish discoloration of the blood and tissues, cyanosis, and hyperemia of the visceral and peripheral blood vessels.

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PART II. GASTRIC ULCER COMPLEX

INTRODUCTION

The increased incidence of gastric ulcers in swine stimulated this investigation on the epizoology, clinical manifestations, gross and microscopic pathology, and mycology. Reports have suggested a relationship of the gastric ulcer complex to gastric hyperacidity, to vitamin A deficiency, to the "eosinophilic hepatitis" problem, and to candidiasis. With the increased use of antibiotic feeds, some reports suggested Candida albicans as a possible etiologic agent in the gastric ulcer complex. Other reports regarded it as a secondary invader.

In rats, prednisolone is known to produce ulcers which are similar to the peptic and steroid-induced ulcers in man. Since this steroid is a powerful gluconeogenic drug, the stress of increased metabolism following injection may simulate the metabolic stress in rapidly growing pigs.

REVIEW OF LITERATURE

A large volume of literature has accumulated on the various aspects of gastric ulcers and prednisolone therapy. An attempt has been made to review selected references referring primarily to swine; however, a few references relating to prednisolone-induced ulcers in rats are included.

Gastric Ulcers in Swine

Association with Infections

Rosenow (1923) was one of the first to report a study of stomach ulcers in swine, calves, cows, sheep, and dogs. Although many different organisms were cultured from the stomachs, Streptococcus spp. were isolated in pure culture or predominating numbers from many of the ulcers. Jensen and Frederick (1939) reported on an 8-year survey of the stomach ulcers of slaughter-age swine at a packing plant, and again, species of Streptococcus were considered as the etiologic agents.

Dodd (1960), in New Zealand, described an ulcerative gastritis and an anemia condition caused by Hyostrongylus rubidus. It was clinically characterized by a retarded growth rate and diarrhea. This condition most seriously affected the adult breeding sows, and many deaths resulted from hemorrhage from 1 or more deep gastric ulcers.

Association with Nutritional Deficiencies

Obel (1953), while studying toxic liver dystrophy in swine, discussed cardial stomach ulcers as a cause of fatal gastrorrhagia. Although the stomach lesion was not believed to be characteristic of the liver disease, a slightly higher incidence of ulcers was noted in experimental groups

fed high levels of cod liver oil. In some groups the incidence of cardial ulceration was 22%, and this was attributed to unsaturated fatty acids and peroxide formation in the absence of sufficient vitamin E.

Hvidsten (1955), while studying the effects of nitrites in pig rations, described hemorrhages in the stomachs of pigs fed high levels of sodium nitrite and cod liver oil. This author reported a lack of vitamin A deposition in the liver.

Pallaske (1960) and Nieberle and Cohrs (1962) described parakeratotic lesions of the cardial region of the stomach and related these lesions to vitamin A deficiency or to toxicoses related to a vitamin A deficiency.

Barron and O'Brien (1963), in Ireland, observed a similar syndrome. In 2 fatal cases, the livers contained 34.8 and 58.9 I.U. of vitamin A/Gm., which they considered suggestive of a deficiency. At necropsy the large intestines contained blood-stained ingesta and the stomachs were ulcerated in the cardial area. They also found keratinization of the nonglandular portion of the stomach which was described as thickened, rough, corrugated and yellowish-brown in color.

Incidence Studies

Jensen and Frederick (1939) in their 8-year study of slaughter-age swine reported the rate of ulcers in the 20,000 pig stomachs examined as 5%, and the highest incidence occurred in the late winter and spring months. Kernkamp (1945) investigated the incidence of ulcers in Minnesota and found 2.38% of 754 swine affected. Berg (1960) discussed a similar ulcer problem at the Iowa Boar Testing Station and indicated a definite increase in the incidence of gastric ulcers in swine from 1956 to 1960.

Perry (1962) reported a 25% incidence of gastric ulcers in 164 swine slaughtered at the Indiana Swine Evaluation Station but found no breed or area predisposing factors. In his experimental studies at the Purdue Agricultural Experiment Station, stomach ulcers were found in more than 50% of the swine, with the highest incidence occurring in swine used to test the value of cooked or gelatinized corn as a component of growing and finishing rations. In contrast, no ulcers were found in the stomachs of pigs not fed the heat-treated corn. Therefore, he concluded that heat treatment of corn, as in pelleting, was a contributory factor in the development of gastric ulcers. In a later report, Perry et al. (1963) discussed their previous work and described a subsequent experiment using gelatinized rations, and they reported a 53% incidence of gastric ulcers. Although the pigs fed raw corn had no stomach ulcers, supplemental thiamine, vitamin B_{12}, l-lysine, or fish meal failed to alter the incidence in pigs fed gelatinized rations.

Clinical and Pathologic Findings

Bullard (1951) published a brief clinical description of esophagogastric ulceration in a large boar in which the ulcer was described as a round lesion, $3\frac{1}{2}$ inches in diameter, surrounding the esophageal orifice which resulted in a secondary anemia and, finally, death.

Kowalczyk (1958), at the University of Wisconsin, described the clinical symptoms and gross pathology of 6 cases of fatal gastrorrhagia in swine during a 2-year period. Although some were indoors and others on pasture, all were fed well-balanced rations. Two to 3 weeks before death the animals appeared to lose weight. Later, vomition of blood and bloody stools were observed. At necropsy the stomachs were filled with fresh and clotted blood.

In a more elaborate report, Kowalczyk et al. (1960) described the stomach ulcers found in swine at the University of Wisconsin farms during 1957 to 1959. During the summer of 1957, several cases of fatal gastrorrhagia occurred in 3- to 5-month-old Chester White and Duroc swine. These animals died suddenly and at necropsy had distended stomachs filled with free and clotted blood and ulcerations in the glandless region of the cardia and in the fundus. They also described a case in an 8-month-old Hampshire boar in which the stomach was filled with blood clots and tarry ingesta was present in the intestine. Besides 1 large ulcer, found in the glandless region of the stomach, others were present in the fundic region and the torus pyloricus.

During this 1957 to 1959 period, a series of experiments was conducted to determine factors in the development of parakeratosis. Twelve of the 161 swine died of acute stomach hemorrhage and 15 additional pigs had stomach ulcers when the swine were slaughtered. In a subsequent experiment using 35 swine, 8 died of acute stomach hemorrhages; and at slaughter 15 of the remaining 27 had stomach ulcers. However, in a later experiment with 56 weaned pigs fed similar rations, none died of gastrorrhagia. At slaughter, 5 of the animals had slight erosions in the glandless area of the cardial region. These workers also reported unsuccessful attempts to reproduce stomach ulcers by intravenous injection of microorganisms isolated from the base and edges of naturally occurring ulcers. Supplements of zinc, copper, and cadmium neither caused nor prevented the ulcers. A seasonal factor was suggested to have an etiologic role.

The pathogeneses of the lesions were described in detail. In the early stages, the stratified squamous epithelium proliferated with subsequent necrosis of the mucosa. The acute inflammation was manifested by congestion of the adjacent mucosa and submucosa, and the capillaries and small blood vessels were prominent. A cellular reaction of neutrophils and a few lymphocytes formed the zone of demarcation. As the necrotic process continued, the ulcer deepened, the walls of the capillaries and small vessels were destroyed, and blood oozed into the lumen. Small thrombi developed in the adjacent vessels, and a more pronounced leukocytic infiltration occurred. As the process of necrosis subsided, lymphocytes and eosinophils infiltrated the lesion and the fibroblasts became more prominent. If fatal hemorrhage did not occur, fibrosis at the base of the ulcer was evident. The lesions in the glandular and nonglandular portions of the stomach were described simultaneously, indicating their apparent close relationship.

Rothenbacher et al. (1963) described the stomach ulcer-gastrorrhagia syndrome in 70 Michigan pigs which died of stomach hemorrhages. This report distinguished between the lesion found in the cardial portion of the stomach and the peptic type ulcer found in the glandular region. In the early or subclinical stage, the cardial lesion was characterized as a parakeratotic proliferation of the squamous epithelium. This was followed by progressive surface necrosis, erosion, ulceration and hemorrhage. Histologic changes in the malpighian and basal layers included edema of the intercellular spaces with microvesiculation, hydropic degeneration, and a lack of keratinization. Although the fibrinonecrotic membranes were invaded by microbial organisms, including cocci, bacilli,

and fungi, they were considered secondary invaders, since these microorganisms were not found in the submucosa. These authors compared this ulcer-gastrorrhagia syndrome to various toxicoses or deficiency syndromes, such as parakeratosis or a vitamin A deficiency. Since many of the pigs were fed an all-plant ration, the possibilities of nitrite or other toxic agents as etiologic factors were postulated. A seasonal variance in incidence was also observed.

Curtin et al. (1963) characterized the clinical and pathologic aspects of the esophagogastric ulcers in swine at the Indiana Swine Evaluation Station. Of the 443 pigs examined, 383 (86.45%) had abnormal keratinization of the stratified squamous epithelium of the stomach. Although only 4 died as a result of hemorrhage, ulcerations were observed in 87 (19.64%). Candida albicans was isolated from many of the lesions, but these authors also considered it as a secondary invader. They cited stresses and increased demands for necessary metabolic requirements as a primary etiologic factor. Since more deaths occurred during late spring and early winter months, they also observed a seasonal variance in the incidence. Introduction of diseases into a herd, changes in management, environmental variation, and immunization were incriminated as other stresses. Although sufficient vitamin A was present in the diet to promote rapid growth, they postulated a subclinical deficiency resulting from inhibitory substances or a lack of enzyme systems to function at an accelerated growth rate. Since specific etiologic factors were unknown, they indicated that rapid growth appeared important to the formation of this esophagogastric lesion.

Candidiasis and Ulcers

Kovalev (1947) was one of the first to report clinical candidiasis in pigs. Although the causative organism was Oidium albicans, it was identical to the Monilia albicans, or Candida albicans, as named today. The disease was characterized by lesions on the nose, gums and buccal mucosa, which later resulted in emaciation and death. In addition to local necrosis, he postulated that the organism produced a systemic toxin.

Quin (1952) described moniliasis in swine droves in Canada. Though the clinical picture varied, some droves had persistent diarrhea, encephalitic symptoms, excessive thirst, and a variable death loss. In 1 group of pigs the esophageal-gastric junction was occluded by inflammatory fungal lesions which yielded a pure culture of Monilia. Since death losses stopped when antibiotics were removed from the ration, he concluded that antibiotic residues in the feed were related to this syndrome.

McCrea and Osborne (1957) reported a case of "thrush" caused by C. albicans in an artificially raised pig. After 14 days on a semi-synthetic diet the pig was vomiting and emaciated; and it was later euthanatized. At necropsy, a pseudomembrane was nearly occluding the terminal portion of the esophagus. Although C. albicans was identified from the colon, an organism belonging to the genus Candida was isolated from the esophagus. Histopathologic examination revealed degenerative and necrotic cellular material, neutrophils, and the yeast hyphae. These authors indicated that candidiasis is rarely diagnosed in swine but that it was frequently diagnosed in man and poultry.

Gitter and Austwick (1959) reported mucormycosis and moniliasis in a litter of pigs. In some of the lesions, Candida albicans occurred

concurrently with the mucormycotic organisms. But C. albicans were present only in the stratified squamous epithelium in the stomach; the mucoraceous hyphae were present only in the glandular portions of the stomach. In the lesions attributed to C. albicans, histopathologic examination revealed necrotic and degenerative processes in the epithelium and yeast cells and pseudohyphae in the cellular debris. These were accompanied by numerous mononuclear cells and eosinophils in the lamina propria, marked thickening of the muscularis mucosa, and a slight edema of the submucosa. Similar lesions were described in the esophagus, lips and tongue. C. albicans was not only isolated from the lesions but also from the bedding, drinking water, and air in the pigs' pens. These isolations indicated the possibility of exogenous transmission, a concept of great importance in the study of both human and animal candidiasis.

Osborne et al. (1960) reported this disease in 41 artificially raised pigs, 25 of which died of the infection. The clinical signs included dullness, anorexia, and diarrhea; and the mouth, tongue, hard palate and stomach contained lesions. Although the stomach lesions were in general less severe, occasionally the superficial mucosa was eroded. Degeneration and necrosis of the stratified squamous epithelium and infiltration of neutrophils, plasma cells, lymphocytes and eosinophils in the submucosa were noted microscopically. Though the epithelial layer contained numerous yeast cells and pseudohyphae, the submucosa was not penetrated. These authors treated the disease successfully with Nystatin, an antimycotic agent. They also discussed the predisposing factor of antibiotic-containing feeds and concluded that the small amount

of antibiotics used in the diet did not alter the normal gut flora sufficiently to predispose to infection by C. albicans.

In Wisconsin, Baker and Cadman (1963) isolated C. albicans from unthrifty pigs, 2 to 8 weeks old, in 8 different herds. One case history involved 160 pigs, 80 of which died of the infection and 30 of which were severely stunted. Despite antibiotic and furazolidone therapy and excellent management and sanitation, losses persisted. The clinical signs included restlessness, inappetence, and vomition. At necropsy, the stomachs were distended with gas and contained a dark green, slimy liquid. The mucosa of the esophageal region, which was thickened and had a rough, scaly, and corrugated appearance, was easily removed and had shallow ulcerations and inflammation. The desquamated epithelium contained numerous mycelial fragments and yeast cells of C. albicans. Although the mortality and morbidity rates were not as high in the other 7 case histories described, the clinical signs and necropsy findings were similar. These workers concluded that C. albicans had a predilection for the esophageal region of the stomach and for the lower portion of the esophagus. Since it was not known whether C. albicans was capable of infecting normal pigs, they also concluded that this problem may have been secondary or incidental to other existing conditions.

An anonymous author (1963) reported a high incidence of fungi in the stomachs of swine in which gastric ulcers were observed. Of more than 600 animals examined, 13.5% had gastric ulcers and 97% of these were located in the esophageal region. Candida albicans was isolated from 47.3% of the ulcers and Candida spp. from 33.3%. In stomachs with no ulcers, but which were not completely normal, C. albicans was isolated from 13.8% and Candida spp. from 21.6%. Moreover, 87% of the stomachs

from which C. albicans was isolated had keratinization of the esophageal region.

Prednisolone-Induced Ulcers in Rats

Nature of Prednisolone

Beckman (1958) described prednisolone as a synthetic dehydrogenated analogue of hydrocortisone. In addition to being one of the most powerful glucocorticosteroids, prednisolone is used to correct adrenal insufficiencies, to inhibit inflammatory reactions, and to counteract allergies.

Prednisolone-Ulcer Relationship

Robert and Nezamis (1958) referred to the development or reactivation of gastroduodenal ulcers in man as a serious side effect of corticosteroid therapy. Thus, a method of producing similar ulcers in experimental animals was available. They produced ulcers in both fasted and nonfasted rats with steroids; however, in the nonfasted group it required 10 to 15 days of treatment with high doses. The steroid-induced ulcers were always found in the glandular portion of the stomach in rats and resembled the human peptic ulcers. These were characterized by hemorrhages, necrosis of a segment of the mucosa and formation of a crater that was composed of necrotic debris and leukocytes. Although these ulcers were usually multiple in number, they never perforated the stomach wall.

These authors also reported that steroids prevented the appearance of ulcers in the forestomachs of fasted rats or rats with a ligated pylorus. The usual ulcerated lesion in the forestomach was an inflammatory reaction initially caused by concentrated gastric juice. This

lesion was characterized first by a tremendous edema of the forestomach wall and an infiltration of inflammatory cells. Since the blood vessels were compressed by the edema, a state of malnutrition of the surface epithelium occurred. Necrosis resulted because the malnourished tissue was not resistant to gastric juices. Steroids prevented the initial inflammatory reaction; there was no edema or ischemia, and the mucosa retained its integrity.

In a subsequent paper, Robert and Nezamis (1963) reported the effects of prednisolone on mucus formation. Using fasted adult Sprague-Dawley rats, 5 mg. of prednisolone was given daily subcutaneously for periods from 1 to 6 days. From their findings of decreased gastric juice acidity, they concluded that steroid ulcers were not related to hyperacidity. They reported, however, a significant decrease in gastric mucus in the steroid-treated rats. From these findings of decreased mucus, the appearance of steroid-induced ulcers was attributed to the corticosteroids, rendering the mucosa more sensitive to the digestive and irritating properties of gastric juices by weakening the mucous barrier. This latter explanation of the pathogenesis of steroid-induced ulcers overshadowed the widely accepted theory that the ulcers were related to hyperacidity.

In summary, the steroid-induced ulcers in rats were limited to the glandular region of the stomach and were related directly to the decreased gastric mucus rather than hyperacidity.

OBJECTIVES

The specific objectives of this research were:

1. To determine the nature of the stomach ulcer problem with respect to (A) the incidence, (B) the role of vitamin A deficiency, (C) gastric hyperacidity, (D) the eosinophilic hepatitis complex, and (E) the role of Candida albicans.

2. To observe and record gross and microscopic lesions associated with the gastric ulcer syndrome, candidiasis, and steroid-induced ulcers in pigs.

3. To determine the pathogenicity of Candida albicans when given by various routes of administration to week-old normal pigs.

4. To attempt to produce ulcers by giving high levels of prednisolone intramuscularly to pigs.

MATERIALS AND METHODS

Experimental Animals

The pig was used as the experimental animal because it was (1) the species in which the stomach ulcers were observed naturally and from which *Candida albicans* was isolated, and (2) available for this research.

Facilities and General Information

From December 1, 1962, to June 30, 1964, the data related to the clinical study of stomach ulcers were accumulated from 1071 pigs slaughtered at Michigan State University Meats Laboratory. The average age of these pigs was 5 to 6 months, the average weight was 200 pounds, and all appeared clinically normal on antemortem inspection.

Experiment I consisted of an epizoological study of 3 groups of pigs: (1) from the Michigan State University Swine Farm and their experiments; (2) from the Swine Evaluation Station at Michigan State University; and (3) from all over Michigan that were entered in various 4-H, FFA, and other shows which required carcass data. In addition to these 1071 pigs, 96 pigs were fed diets varying in vitamin A and carotene content, and at slaughter the stomachs were examined.

Experiment II consisted of 12 pigs used in a study of candidiasis. These were maintained in rooms 57 and 58, Giltner Hall, Michigan State University. Assignment to various treatments was made by using random numbers (Dixon and Massey, 1957).

Experiment III, Trials 1 and 2, consisted of 24 pigs used in the study of the prednisolone-ulcer syndrome. In Trial 1, 4 pigs were

purchased from a nearby farm and maintained at the Veterinary Research Farm. In Trial 2, the 20 pigs that were used were raised at the Michigan State University Swine Farm.

Analytical Procedures

Hematology. Blood samples were collected from the anterior vena cava, using ethylene diaminotetraacetic acid (EDTA) or heparin as an anticoagulant. The samples were collected at selected intervals, depending on the experiment. Hemoglobin was determined by the cyanmethemoglobin method, packed cell volume values by the micro method (capillary tube), and total white blood cell counts were made using Turk's diluting fluid and the hemacytometer. At the time blood samples were collected, blood smears were made, then stained with Wright's stain and a differential white blood cell count was determined.

Histopathology. Tissue sections were collected, preserved in buffered 10% formalin, and were stained with hematoxylin and eosin. Selected sections from Experiments I and II were stained by the Gomori's silver methenamine method to demonstrate fungi, and selected sections from Experiment III were preserved in Carnoy's fluid and stained by the Best's carmine method to demonstrate glycogen. These procedures were according to the United States Armed Forces Institute of Pathology Manual of Histologic and Special Staining Technics (1957).

Mycologic Examinations

Sabouraud's glucose agar with 20 units of penicillin and 40 units of streptomycin added per ml. was used for the isolation of C. albicans in Experiments I and II. For identification of the typical chlamydospores, "Difco" cornmeal agar was used (Beneke, 1962).

Specific Procedures for Each Experiment

Experiment I (Nature of Ulcer Problem)

These pigs were fasted approximately 18 hours prior to slaughter. Using an ice tong type electric immobilizer (Valley Forge Research, Inc.), the pigs were stunned by a humane method. Then they were exsanguinated by severing the large vessels anterior to the heart. After death, the carcasses were scalded at 142 F. and dehaired. The average time lapse from stunning to evisceration was 30 minutes. After evisceration, the viscera were examined according to routine post-mortem procedures. Then the stomach was opened by incising along the greater curvature, which allowed a careful gross examination of the mucosal surface and the wall. The stomachs were classified as (1) normal, (2) keratinization of the esophageal region, (3) ulceration of the esophageal region, and (4) ulceration of the fundic region.

The pH values of 167 stomachs were determined with a Beckman Glass Electrode pH Meter - Model H-2 using a Beckman 39142 Combination Electrode (Beckman Instruments, Inc., Fullerton, Calif.). These values were determined in the esophageal region and were recorded after inserting the electrode 1 cm. into the esophageal orifice. The pyloric values were obtained with the electrode opposite the torus pyloricus.

Samples for mycologic examination were collected from 10 stomachs with esophageal ulcers and 10 with keratinized lesions. The lesions were swabbed, streaked on Sabouraud's pen-strep agar, and incubated at 22 C. for 72 hours. The characteristic colonies were removed, streaked on cornmeal agar and incubated. C. albicans was identified by means of its typical chlamydospores.

Experiment II (Candidiasis)

Twelve Yorkshire crossbred baby pigs were born April 17, 1963. At 5 days of age, the pigs were given 2 ml. of an iron preparation (Armidextran, Armour & Co.) intramuscularly. At 6 days of age, the pigs were weaned, ear notched and weighed, and blood samples and rectal swabs were collected.

The 12 pigs were randomly selected and put into 5 groups, 3 groups of 2 each and 2 groups of 3 each. The ration consisted of homogenized, pasteurized, whole milk and eggs (1 egg per quart of milk), and mixed by a Waring Blendor. The pigs were fed 3 times a day ad libitum.

Candida albicans, type B, were grown on Sabouraud's medium. The colonies were then removed and placed in physiological saline. With a hemacytometer, the yeast cells were counted and the average per ml. was calculated as 357,000 organisms. Except for the controls, each pig was given 1 ml. of this stock culture. The oral administration was in 20 ml. of the milk-egg mixture at feeding time, the intravenous injection was made into the anterior vena cava, and the intraperitoneal injection was made into the right lower abdominal region. In the remaining 2 pigs the foramen magnum was located, and with a 2-inch, 20-gauge needle, the 1 ml. was inoculated into the subarachnoid space. Blood samples were collected at selected intervals using EDTA as an anticoagulant, rectal swabs were taken, and weights were recorded twice per week. The experiment lasted 21 days. The pigs were then euthanatized with electricity and carefully observed for gross changes at necropsy. Specimens from several vital organs were collected for histopathologic examination.

Experiment III (Prednisolone)

Trial 1. Four Yorkshire crossbred pigs, 8 weeks of age, were divided into 2 groups of 2 each. Using 1 group as the control, the other 2 pigs were given 100 mg. of prednisolone* intramuscularly daily for 7 days. Feed was withheld the last 72 hours for both groups, and the pigs were necropsied 12 hours after the last injection. Gross observations were recorded and selected specimens were preserved for histopathologic examination.

Trial 2. Twenty crossbred pigs were divided into 2 groups of 10 each. One group of 10 was used as controls. The experimental group of 10 was subdivided into 2 groups of 5 each. One group was given 100 mg. of prednisolone intramuscularly per pig per day for 7 days, and the other group was given 200 mg. in a similar manner. Feed was withheld 27 hours before slaughter, and the pigs were slaughtered 12 hours after the last injection. Weights were recorded at selected intervals. At slaughter, the tissues were examined for gross lesions, selected sections were preserved for histopathologic examination, and the weights of the livers, hearts, stomachs, and adrenal glands were recorded.

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*Delta-Cortef, Sterile Aqueous Suspension, the Upjohn Company, Inc., Kalamazoo, Michigan.

RESULTS

Experiment I (Nature of Ulcer Problem)

Incidence and Epizoology

The incidence of stomach lesions was determined in 1071 slaughter-age pigs which included 673 pigs from the Michigan State University Swine Farm, 170 from the Swine Evaluation Station, and 228 from outside sources. The data regarding the monthly incidence of lesions are given in TABLE 2-1. Data summarizing the source of the pigs and incidence of stomach lesions are presented in TABLE 2-2. Although all the pigs appeared clinically normal on antemortem inspection, 39 of the 115 with esophageal ulcers had free blood in the stomachs on examination at slaughter. The highest incidence of all types of stomach lesions occurred in the pigs from the Swine Evaluation Station, and the highest incidence of ulcers occurred during the months from December to April.

Role of Vitamin A

Stomachs of 97 pigs from a vitamin A experiment were examined at slaughter to determine the role of vitamin A. These pigs were fed a basal ration low in vitamin A and the same ration with varying levels of carotene and vitamin A supplemented. The information regarding the incidence of stomach lesions in pigs fed various amounts of vitamin A and carotene is given in TABLE 2-3. No major differences were noted in the incidence of stomach lesions among the different groups; however, only 9 of the 97 stomachs examined were grossly normal.

TABLE 2-1.--Monthly Incidence of Stomach Lesions in Slaughtered Pigs.

Month	Total Swine			Keratinization			Esophageal Ulcer			Fundic Ulcer		
	*SF	SE	0	SF	SE	0	SF	SE	0	SF	SE	0
December (1962)	54	7	0	14	6	0	11(2)**	5(2)	0	5	0	0
January	80	12	59	9	10	6	5(2)	9(3)	2	0	2	1
February	20	25	2	4	10	0	4(1)	8(2)	0	1	2	0
March	7	10	0	2	8	0	2	2(2)	0	1	1	0
April	32	5	0	3	0	0	3(1)	5(3)	0	0	0	0
May	29	0	0	7	3	0	5	0	0	4	0	0
June	20	3	0	4	2	0	2(1)	0	0	2	1	0
July	57	28	0	30	18	0	3(3)	4(2)	0	1	5	0
August	11	21	18	8	19	1	1(1)	4(1)	0	3	1	0
September	31	6	7	22	7	2	1(1)	1	0	2	1	0
October	29	3	7	6	2	3	1	0	0	0	0	0
November	45	10	4	11	5	0	0	0	0	0	0	0
December	47	16	2	12	13	2	1	4(1)	0	1	1	0
January	34	12	0	24	4	0	7(3)	2(1)	0	1	0	0
February	9	6	104	3	4	14	1	2(1)	2(2)	0	0	0
March	76	1	9	34	1	9	7(2)	0	1	2	0	0
April	64	2	16	15	1	4	5(2)	0	0	0	0	0
May	28	3	0	9	2	0	4	1	0	0	0	0
TOTAL	673	170	228	222	115	41	63(19)	47(18)	5(2)	23	14	1

*SF (Swine Farm): SE (Swine Evaluation): 0 (Other Origins).

**( ) Number of pigs having free blood in stomach.

TABLE 2-2.--Incidence of Stomach Lesions in Slaughtered Pigs from Various Origins.

Origin	No. of Pigs	Stomach Lesions		
		Keratinization	Esophageal Ulcer	Fundic Ulcer
Swine Farm	673	222	63	23
%		33.0	9.6	3.4
Swine Eval.	170	115	47	14
%		67.7	27.7	8.2
Other	228	41	5	1
%		18.0	2.2	0.4
Total	1071	378	115	38
%		35.3	10.7	3.5

TABLE 2-3.--Incidence of Stomach Lesions in Pigs on Basal Rations Low in Vitamin A With Various Levels of Carotene or Vitamin A Supplemented.

Group No.	No. of Pigs	Ration	Normal	Stomach Lesions		
				Keratinization	Esophageal Ulcer	Fundic Ulcer
1	9	Basal	2	7	0	0
2	44	Carotene added	4	34	3	3
3	44	Vitamin A added	3	38	1	2
Total	97		9	79	4	5

The Role of pH

The pH values were determined in stomachs from the 97 pigs of the vitamin A experiment and 70 pigs fed a standard swine ration. The average pH values of the esophageal and pyloric regions of the stomachs and the relationship to the gross lesions are given in TABLE 2-4. The average pH value in the esophageal region of normal stomachs was 2.5 and in the pyloric region, 3.5. In contrast, the average pH value was 3.8 in the keratinized esophageal regions, 5.2 in the esophageal region of the stomachs with fundic ulcers, and 6.2 in the ulcerated esophageal regions. Although free blood was present in 3 of 9 stomachs, the pH range for all stomachs with ulcers in the esophageal region was 6.2 to 6.6. In general, the highest pH values were recorded in stomachs containing free blood.

Relation to Eosinophilic Hepatitis Complex

The data on liver condemnations from 1305 pigs slaughtered between August 1, 1962, and June 1, 1964, are given in TABLES 2-5 and 2-6. The highest incidence of liver condemnations for eosinophilic hepatitis occurred from September through January of each year and occurred from swine raised at the Swine Evaluation Station. The incidence of stomach ulcers and eosinophilic hepatitis in the same animal was lower than the naturally occurring incidence of either (TABLES 2-2 and 2-5).

Gross and Microscopic Lesions

Keratinization. Keratinization was characterized grossly by a proliferation of the squamous epithelium of the esophageal region of the stomach. This proliferation appeared as bright yellow to brownish-yellow plaques,

TABLE 2-4.--Average pH Values of Stomachs from (A) Pigs Fed Rations Varying in Carotene and Vitamin A, and (B) Pigs fed Standard Rations.

Stomach Lesions	Average pH	
	Esophageal Orifice	Pyloric Orifice
A. Pigs fed rations varying in carotene and vitamin A		
Normal (9)*	2.6	3.5
Slight keratinization (23)	3.4	4.3
Severe keratinization (56)	4.3	4.9
Total keratinization (79)	3.7	4.5
Esophageal ulcer (4)	6.1	6.2
Fundic ulcer (5)	5.0	5.8
Total (97)	3.4	4.3
B. Pigs fed standard rations		
Normal (34)	2.5	3.5
Keratinization (30)	3.9	4.7
Esophageal ulcer (5)	6.3	6.5
Fundic ulcer (1)	5.7	5.8
Total (70)	2.8	3.8
C. Composite of A and B		
Normal (43)	2.5	3.5
Keratinization (109)	3.8	4.6
Esophageal ulcer (9)	6.2	6.3
Fundic ulcer (6)	5.0	5.8
Total (167)	3.0	4.0

*( ) Number of stomachs examined.

TABLE 2-5.--Incidence of Liver Condemnations in Slaughtered Pigs from Various Origins.

Origin	No. of Pigs	Eosinophilic Hepatitis	Ascarid Scars	Eosinophilic Hepatitis and Ulcers
Swine Farm	856	137	26	13
%		16.0	3.0	1.5
Swine Eval.	221	57	8	7
%		25.6	3.6	3.2
Other	228	33	38	2
%		14.5	16.7	1.0
Total	1305	227	72	22
%		17.4	5.5	1.7

TABLE 2-6.--Monthly Incidence of Liver Condemnations from Slaughtered Pigs.

Month	Total Swine			Eosinophilic Hepatitis			Ascarid Scarring			Ulcers and Eosinophilic Hepatitis		
	SF*	SE	O	SF	SE	O	SF	SE	O	SF	SE	O
August (1962)	48	28	0	0	11	0	1	2	0	0	1	0
September	74	8	0	14	1	0	3	1	0	2	0	0
October	36	15	0	15	4	0	0	1	0	1	0	0
November	25	0	0	10	0	0	1	0	0	0	0	0
December	54	7	0	11	3	0	0	0	0	5	1	0
January	80	12	59	9	2	3	5	0	9	1	1	1
February	20	25	2	1	1	0	0	0	0	0	0	0
March	7	10	0	1	1	0	0	2	0	0	0	0
April	32	5	0	0	4	0	1	0	0	0	2	0
May	29	0	0	6	0	0	1	0	0	0	0	0
June	20	3	0	4	1	0	0	0	0	0	0	0
July	57	28	0	4	12	0	0	0	0	0	1	0
August	11	21	18	0	7	3	3	2	5	0	0	0
September	31	6	7	10	2	4	0	0	0	0	0	0
October	29	3	7	7	0	0	1	0	0	0	0	0
November	45	10	4	13	3	0	1	0	0	0	0	0
December	47	16	2	13	3	0	0	0	0	0	0	0
January	34	12	0	10	0	0	0	0	0	2	0	0
February	9	6	104	0	0	14	0	0	24	0	0	0
March	76	1	9	3	0	9	0	0	0	0	0	1
April	64	2	16	4	0	0	8	0	0	2	0	0
May	28	3	0	2	2	0	1	0	0	0	1	0
TOTALS	856	221	228	137	57	33	26	8	38	13	7	2

*SF (Swine Farm): SE (Swine Evaluation): O (Other Origins).

and the extent of keratinization varied from small areas to the entire esophageal region. Fig. 2-1 illustrates moderate to heavy keratinization.

Histopathologic examination of these keratinized lesions indicated a parakeratotic proliferation of the squamous epithelium. Below the outer layers of nucleated epithelial cells, a 5- to 8-cell layer had ballooning degeneration. The malpighian layer was hyperplastic, similar to acanthosis. Even though fungal hyphae were often seen in the superficial layers of these lesions, little to no inflammatory reaction was noted. Fig. 2-2 illustrates uniform parakeratosis and acanthosis, and Fig. 2-3 illustrates parakeratotic plaque formation.

Esophageal ulcers. Nearly all esophageal ulcers were accompanied by moderate to extensive keratinization of the squamous epithelium. Grossly, these ulcers varied from (1) a single break in the continuity of the mucosa, to (2) numerous bleeding ulcers with clots attached, or to (3) complete loss of epithelium and replacement with granulation tissue. The first type of ulcer was characterized by a separation in the mucosal surface at or near the squamous-glandular epithelial junction (Fig. 2-4). The second type consisted of the first plus hemorrhage (Fig. 2-5). Only a few stomachs contained attached clots, and usually the stomachs were filled with free blood clots and unclotted blood. Although a few of the stomachs contained a small amount of feed, usually no food material was detected. The third type of ulceration consisted of a chronic fibrinonecrotic gastritis of the esophageal region in which no mucosal surface was evident (Fig. 2-6).

Microscopically, the first type of ulcer had parakeratotic proliferation in some areas and complete loss of epithelium in others. The

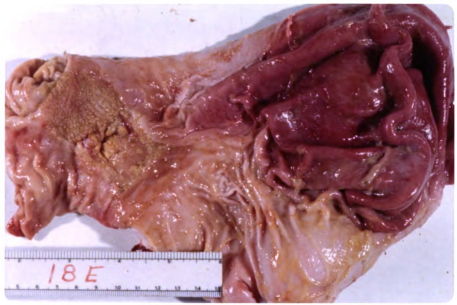

Fig. 2-1.--A stomach of pig with a keratinized esophageal region and adhering mucus in the fundic region.

Fig. 2-2.--Photomicrograph of Fig. 2-1. Parakeratotic proliferation of the squamous epithelium of the esophageal region. H & E stain; x 75.

Fig. 2-3.--Parakeratotic proliferation of squamous epithelium on the esophageal region of the stomach. Note the plaque formation. H & E stain; x 75.

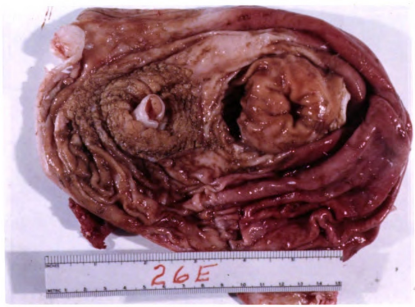

Fig. 2-4.--The stomach of a pig with a keratinized esophageal region. At the squamous-glandular junction, small ulcers are present.

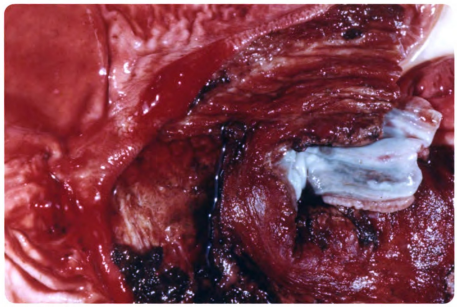

Fig. 2-5.--Esophageal region of a pig's stomach with ulcers and attached blood clots.

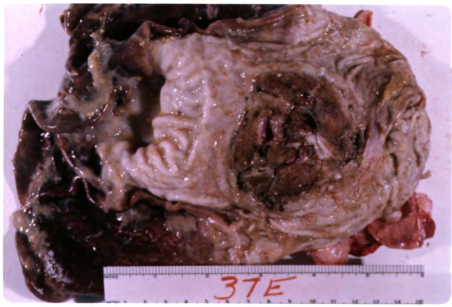

Fig. 2-6.--The stomach of a pig with a fibrinonecrotic lesion in the esophageal region and adhering mucus in the fundic region.

ulcerative lesions consisted of necrotic debris, infiltrations of neutrophils, lymphocytes, and varying degrees of edema (Figs. 2-7 and 2-8). Colonies of bacteria and fungal hyphae were occasionally present on the surface, but rarely invaded the submucosa. The second, or bleeding-type, ulcer contained inflammatory exudates, thrombosis, erythrocytes, and necrotic debris. The third type of ulceration was characterized by numerous lymphocytes, neutrophils and fibroblasts infiltrating the fibrinonecrotic area. No epithelium was present, and usually bacteria invaded the surface of the lesion.

Fundic ulcers. Grossly, these lesions appeared as dark reddish to black ulcers in the fundic region and were surrounded by an area of hyperemia (Fig. 2-9). In different stomachs, these lesions varied in number from 1 to 6 and in average diameter from 1.5 to 70 mm. Microscopically, coagulation necrosis of the glandular epithelium, occasional thrombosis of blood vessels, and erythrocytes on the ulcerated surface were present. The inflammatory response included infiltrations of lymphocytes and neutrophils and proliferation of fibroblasts (Fig. 2-10). No fungal or bacterial organisms were noted in these fundic lesions.

Eosinophilic hepatitis and ascarid-scarred livers. The livers seen on routine post-mortem examination classed as eosinophilic hepatitis contained varying numbers and sizes of lesions which were visible not only on the surface but also in the parenchyma of the liver. Some of the lesions were raised from the surface, causing the capsule to bulge. The affected livers varied from normal in size to greatly enlarged.

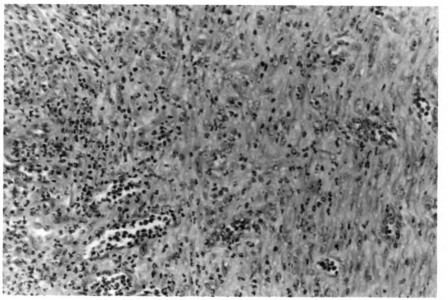

Fig. 2-7.--Submucosa of esophageal region of stomach shown in Fig. 2-4. Note the neutrophils and lymphocytes. H & E stain; x 187.

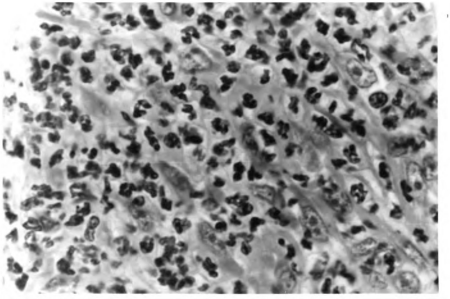

Fig. 2-8.--Higher power of Fig. 2-7. Note the numerous neutrophils and proliferating fibroblasts. H & E stain; x 750.

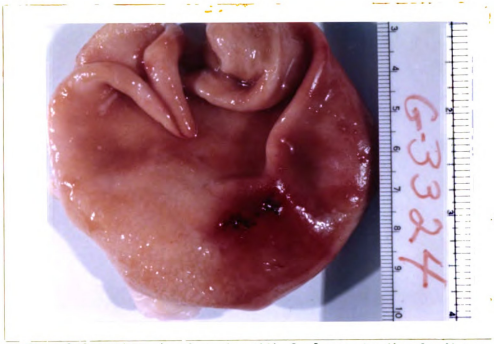

Fig. 2-9.--Stomach of a pig with 2 ulcers in the fundic region.

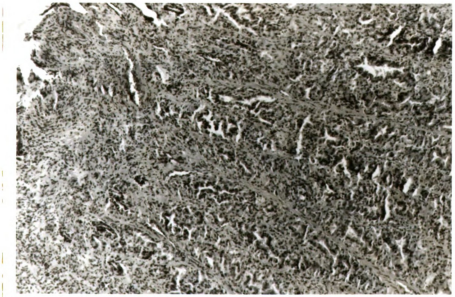

Fig. 2-10.--Photomicrograph of Fig. 2-9. Ulcerative gastritis of the fundic region of stomach. Note coagulation necrosis of the mucosa with infiltrations of inflammatory cells. H & E stain; x 75.

Palpation of the affected livers indicated that the most severely affected were friable. The less extensively affected livers were normal to firm on palpation. The individual lesions were from 1 to 4 cm. in diameter, and the color varied from gray to yellow. Since no tendency toward encapsulation by fibrosis was seen, the lesions appeared proliferative and disseminated into the normal parenchyma. The cut surface of the lesion appeared as yellowish to gray perilobular discoloration with occasional petechial hemorrhages. The hepatic lymph nodes were normal in size to greatly enlarged, the latter being congested and edematous. Fig. 2-11 illustrates a liver with eosinophilic hepatitis. In contrast, grossly, ascarid-scarred livers had whitish foci, primarily on the surface of the liver. These foci were 2 to 5 mm. in diameter and did not extend deeply into the parenchyma (Fig. 2-13). The hepatic lymph nodes were normal, and in each case ascarids were found in the small intestine.

The microscopic examination of the eosinophilic hepatitis lesions indicated acute, subacute, or chronic types. The acute type was characterized by an extensive infiltration of eosinophils and occasional lymphocytes and neutrophils into the periportal areas. Many of the sinusoids and blood vessels near the lesions were distended with blood, and some of the liver cells were undergoing atrophy and necrosis. The subacute type of eosinophilic hepatitis consisted of large numbers of eosinophils, proliferating fibroblasts, and a few lymphocytes in the periportal areas. Many of the lobules were reduced in size due to the atrophy and necrosis of the hepatic cells in the periportal areas (Fig. 2-12). The chronic type of eosinophilic hepatitis was characterized by a

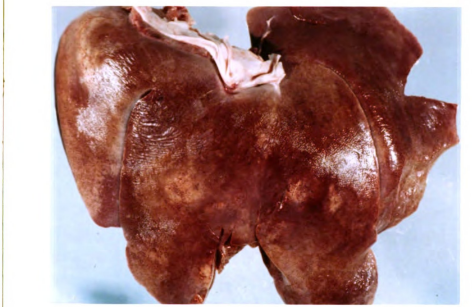

Fig. 2-11.--Porcine liver, weighing 2740 Gm. Note the yellowish nodules and the rounded edges.

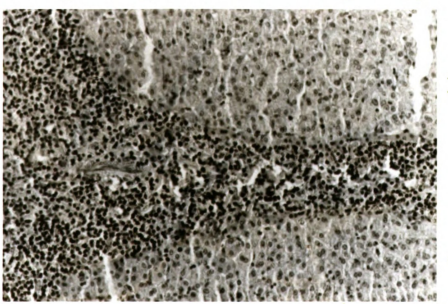

Fig. 2-12.--Photomicrograph of Fig. 2-11. Subacute perilobular eosinophilic hepatitis in a pig liver. H & E stain; x 187.

marked thickening of the interlobular septa, fibroblastic proliferation, and the production of collagen. Again, numerous eosinophils and a few lymphocytes were seen in the septa. Microscopic examination of sections through livers with lesions resembling typical ascarid scarring had a focal hepatitis characterized by a marked increase in lymphocytes and neutrophils. Fibroblastic proliferation and parenchymal cell regeneration were also seen. Some eosinophils were noted in the examination of these lesions (Fig. 2-14).

Mycology

Candida albicans was cultured and identified from 7 of the 10 esophageal ulcerative lesions and from 5 of the 10 keratinized lesions (Fig. 2-15). Microscopic examination of some of the stomachs indicated pseudohyphae in the superficial areas of the parakeratotic lesions (Fig. 2-16).

Experiment II (Candidiasis)

Analyses

Hematology. Hemoglobin and packed cell volume values and total and differential white blood cell counts remained within the normal ranges for the entire experiment in all pigs in each group. Nucleated red blood cells were seen, but in numbers within the normal range for pigs of this age. During the first week of the experiment, a moderate macrocytosis of the red blood cells was noted.

Culture. Rectal swabs were negative for C. albicans by culture on Sabouraud's pen-strep agar, except for 2 of the 3 pigs which were fed the

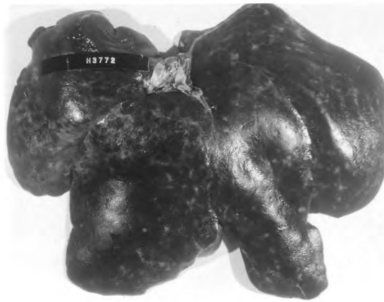

Fig. 2-13.--Liver of a pig which had numerous large ascarids in the small intestine. Note the numerous whitish foci (ascarid scars?) on the surface.

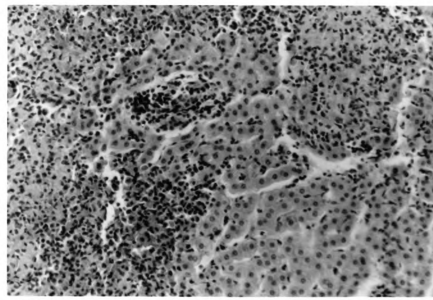

Fig. 2-14.--A disseminating type of lesion in a grossly typical ascarid-scarred liver. Note the necrosis and the infiltration of numerous inflammatory cells. H & E stain; x 187.

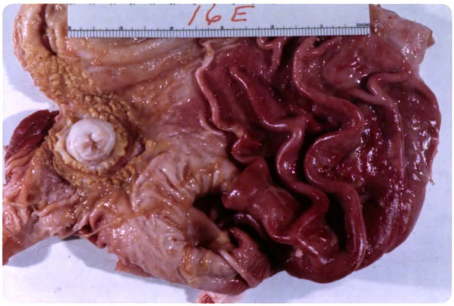

Fig. 2-15.--Yellow keratinized and eroded esophageal region of a stomach of a pig. C. albicans was isolated from this lesion.

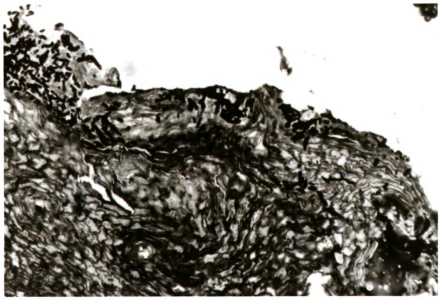

Fig. 2-16.--C. albicans in the mucosal surface of a pig stomach. Gomori's silver methenamine stain; x 187.

organism. At necropsy, C. albicans was isolated from the cerebrospinal fluid in both pigs given the organism in the subarachnoid space. In 1 of the 2 pigs given the organism intravenously, C. albicans was isolated from the lung and liver. In 2 of the 3 pigs which were fed the organism, C. albicans was cultured from the rectum and stomach. The typical chlamydospores for identifying C. albicans are given in Fig. 2-17.

Signs

The daily rectal temperatures of all pigs were within the normal range, except for pigs given C. albicans intravenously. Their temperature increased slightly 24 hours after inoculation and remained elevated for 3 days. Both pigs given the organism intravenously were dyspneic for 4 days after inoculation. On the 4th day 1 of the pigs was weak, unable to rise, and was then euthanatized and necropsied. The 2 pigs given C. albicans in the subarachnoid space had ataxia, depression and weakness for 3 days following inoculation. All other pigs were clinically normal throughout the experiment.

Gross Lesions

The lungs of the pig that was necropsied on the 4th day after intravenous inoculation contained numerous areas of red hepatization scattered throughout all lobes. These lesions were 1 cm. in diameter and gave a speckled or mottled appearance to the lung. The liver contained a few white foci, which were 1 to 3 mm. in diameter and scattered throughout the parenchyma. At necropsy, the remaining pigs had no gross lesions attributable to C. albicans.

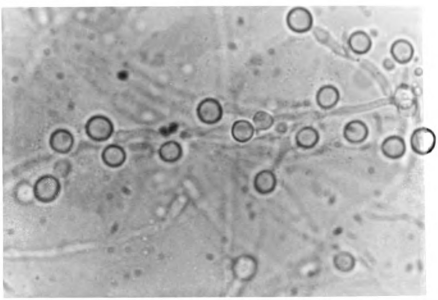

Fig. 2-17.--Typical chlamydospores of C. albicans grown on cornmeal agar. Lactophenol preparation; x 750.

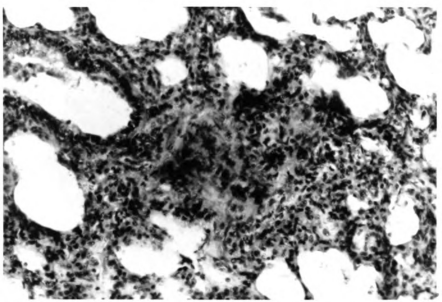

Fig. 2-18.--Granuloma in the lung of a pig given C. albicans intravenously. H & E stain; x 187.

Microscopic Lesions

Microscopically, the tissues of the pig necropsied 4 days after the intravenous injection had the following lesions:

(1). The lung had numerous interstitial granulomatous lesions containing macrophages, lymphocytes, neutrophils, and the fungal organisms (Fig. 2-18).

(2). The liver contained numerous small granulomas made up of macrophages, an occasional giant cell, lymphocytes, neutrophils, and the fungal organisms (Figs. 2-19, 2-20, and 2-21).

(3). The myocardium had a few small granulomatous lesions composed of macrophages, neutrophils, lymphocytes, and the fungal organisms.

(4). The kidney had a few lymphocytes in the interstitial tissue of the cortex, but no organisms.

Two of the 3 pigs given the organism orally had the following lesions:

(1). Erosions of the buccal mucosa containing lymphocytes, neutrophils, bacteria, and a few fungal organisms.

(2). The esophageal region of the stomach had an occasional parakeratotic plaque, lymphocytic infiltration of the mucosa, and a few C. albicans.

The 2 pigs in which C. albicans was given in the subarachnoid space had a mild meningitis, characterized by numerous lymphocytes and the organisms (Fig. 2-22).

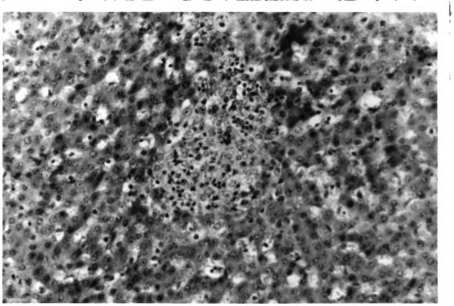

Fig. 2-19.--Granuloma with necrosis in the liver of a pig given C. albicans intravenously. H & E stain; x 187.

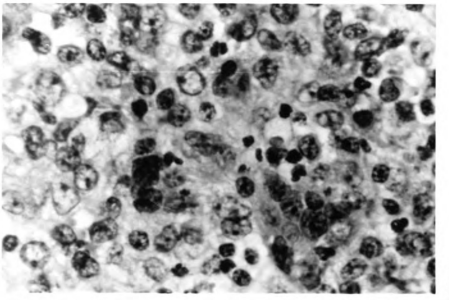

Fig. 2-20.--Higher power of Fig. 2-19. Note the neutrophils, lymphocytes and macrophages. H & E stain; x 750.

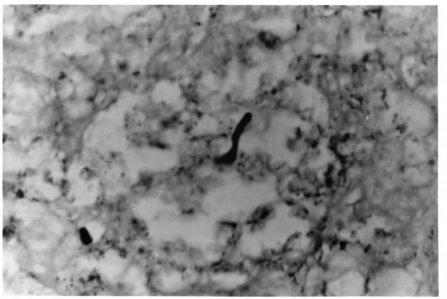

Fig. 2-21.--C. albicans in the granuloma shown in Fig. 2-19. Gomori's silver methenamine stain; x 750.

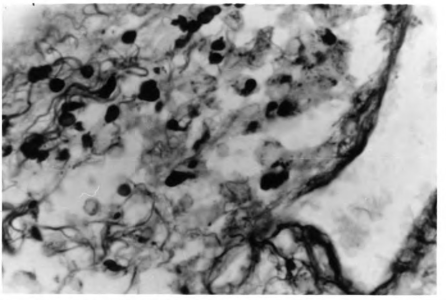

Fig. 2-22.--C. albicans in the meninges of a pig given the organism in the subarachnoid space. Gomori's silver methenamine stain; x 750.

Experiment III (Prednisolone)

Trial 1

Signs

The 2 pigs given 100 mg. of prednisolone daily for 7 days appeared to consume excessive amounts of feed and water. From the 2nd through the 4th day, they had extensive abdominal distention which was probably due to fluid retention. Feed was withheld at the end of the 4th day and the abdominal distention was not apparent the last 2 days of the experiment.

Gross Lesions

At necropsy, the esophageal region of the stomachs of the prednisolone-treated pigs was keratinized and ulcerated. One of the stomachs contained free blood and a clot attached at the ulcer (Figs. 2-23 and 2-24). The fundic region of 1 stomach had numerous small ulcers (Figs. 2-25 and 2-26). The control pigs had keratinization of the stomachs but no ulcers.

Microscopic Lesions

The stomach lesions of the prednisolone-treated pigs had parakeratotic proliferation in some areas, necrosis and complete lack of squamous epithelium in others. Lymphocytes, a few neutrophils, and erythrocytes were present in the necrotic ulcerated area. Examination of the fundic region indicated minute areas of necrosis of the glandular epithelium and infiltration of the necrotic areas by lymphocytes and a few neutrophils.

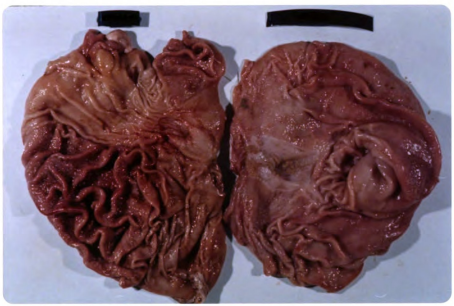

Fig. 2-23.--Stomachs from prednisolone-treated and control pigs. Note the hemorrhage in the esophageal region of the stomach of the prednisolone-treated pig on the left.

Fig. 2-24.--Closeup of Fig. 2-23. Note the clot adhering to the ulcerative lesion near the junction of the esophageal and cardiac regions.

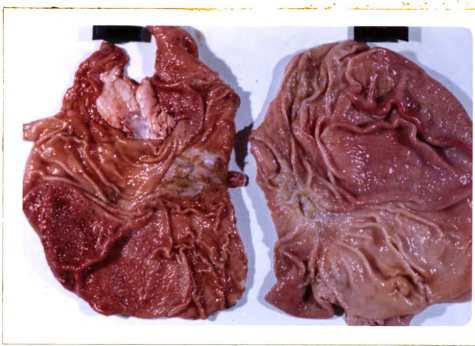

Fig. 2-25.--Stomachs from prednisolone-treated and control pigs. Hemorrhages and ulcerations are present in the fundic region of the stomach of the prednisolone-treated pig on the left.

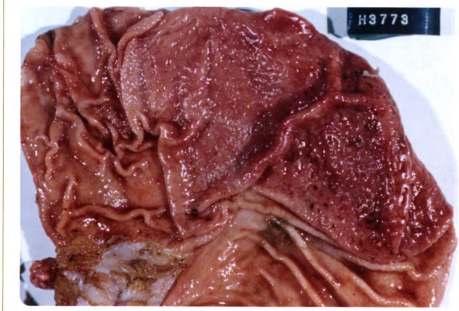

Fig. 2-26.--Closeup of Fig. 2-25. Hemorrhages and ulcerations on the fundic region of the stomach of prednisolone-treated pig.

Trial 2

Signs

No clinical signs of prednisolone toxicity were observed in the 2nd and more detailed study of prednisolone-induced ulcers in pigs. These pigs were considerable larger than the pigs used in Trial 1.

Analyses

Since no major differences were noted in the 2 groups given different amounts of prednisolone, these 2 groups were combined and the analytical data are given in TABLE 2-7. Although major differences were noted in the average weight gains over a 6-day period for the control and treated groups, after a 27-hour shrink prior to slaughter, these gains were lost in the prednisolone-treated pigs. In the treated pigs the average weights of the livers and hearts were greater but the average weights of the stomachs and adrenal glands were lower than those of the controls. No major differences were noted in the average hemoglobin and packed cell volume values, but the average total white blood cell counts and lymphocyte counts were lower, which constituted an absolute lymphopenia in the treated pigs.

Gross Lesions

The data on the incidence of stomach lesions are given in TABLE 2-8. Only 1 esophageal ulcer was noted in the control pigs, in contrast to 8 in the treated pigs. These ulcers were small, and no evidence of intragastric bleeding was noted.

TABLE 2-7.--Experiment III. Trial 2. Average Live Weights, Organ Weights, and Blood Values of Pigs Given Prednisolone Intramuscularly.

	Controls (10 pigs)	Prednisolone-Treated (10 pigs)
Weights (lb.)		
Initial (3-23-64, 2 p.m.)	219	208
Final (3-29-64, 6 a.m.)	229	225
Gain (6 days)	10	17
At slaughter (3-30-64, 9 a.m.)	217	207
(Shrink 27 hours)	12	18
Weights (Gm.)		
Liver	1374	2058
Heart	307	368
Stomach	690	600
Left adrenal	2.0	1.5
Hb. (Gm./100 ml.)	15.4	16.1
PCV (%)	45	46
WBC (cells/cmm.)	15,900	11,100
PMN	34	59
Stab.	0	1
Lymph.	62	35
Mono.	2	4
Baso.	0	0
Eosin.	2	1

TABLE 2-8.--Experiment III. Trial 2. Incidence of Stomach Lesions in Pigs Given Prednisolone Intramuscularly.

	Controls (10 pigs)	Prednisolone-Treated (10 pigs)
Keratinization	8	8
Esophageal ulcers	1	8
Fundic ulcers	1	2

Microscopic Lesions

Microscopic examination of the prednisolone-induced ulcers indicated necrosis of the surface epithelium, a slight infiltration of neutrophils and lymphocytes, and a slight edema. The inflammatory response in the fundic ulcers was also minimal as compared to the control. Other interesting findings accompanying the ulcer syndrome in the prednisolone-treated pigs were extensive glycogen deposits in the liver and heart, as demonstrated by the Best's carmine stain, and atrophy of the adrenal cortex.

DISCUSSION

A high incidence of gastric ulcers in pigs was indicated by the data from a total of 1071 pigs, of which 378 (35.3%) had abnormal keratinization of the stomachs, 115 (10.7%) had esophageal ulcers, and 38 (3.5%) had fundic ulcers. The information on the pigs from the Swine Evaluation Station that had the following incidence of lesions - 67.7% with keratinization, 27.7% with esophageal ulcers, and 8.2% with fundic ulcers - agrees closely with the Purdue University report of Perry (1962), who recorded 25% incidence of esophageal ulcers, and of Curtin et al. (1963), who gave a 20% incidence. The data reported herein indicate a lower incidence of ulcers in the pigs from the Swine Farm and outside sources than those from the Swine Evaluation Station. Although in many cases the rations were similar, the pigs from the evaluation station were subjected to environmental changes when transported from the farms to the station. This is suggested as a possible stress factor for the pigs having the highest incidence of stomach ulcers as compared to pigs raised to slaughter weight in a constant environment. Recently, Muggenburg et al. (1964b) reported a 16% incidence in farm raised swine in Wisconsin and a 7% incidence in Iowa and Illinois. The data in this paper, along with the report by Berg (1960) at the Iowa Boar Testing Station, indicate that the highest incidence of ulcers occurs in production programs that strive for top feed efficiency and growth rate. Regarding seasonal variance in incidence, the work here indicated the highest incidence of ulcers was from December to April. This is somewhat in contrast to the report of Curtin et al. (1963),

who reported more deaths due to ulcers in the early winter and late spring months.

The pathogenesis and gross and microscopic pathology of the stomach lesions were similar to those described by Kowalczyk et al. (1960), Rothenbacher et al. (1963), and more recently in a detailed report by Muggenburg et al. (1964b). The stomach lesions appear to be initiated with a parakeratotic proliferation of the squamous epithelium, followed by necrosis and later ulcerations. The esophageal ulcer and fundic ulcer appeared as separate and unrelated entities from both the epizoologic and pathologic standpoints.

In the experiment on feeding rations containing varying levels of vitamin A and carotene, there were no major differences in the incidence of stomach lesions. This information would not support the suggestion of Rothenbacher et al. (1963) and Curtin et al. (1963), who postulated that a subclinical vitamin A deficiency was an underlying etiologic factor in the gastric ulcer complex. The work described previously in Part I of this thesis indicated that prolonged nitrite ingestion was not associated with the incidence of stomach ulcers. This does not confirm the suggestions offered by several workers.

Hyperacidity of the stomach was not incriminated as a possible etiologic factor. Since the average pH value in the esophageal region of normal stomachs was 2.5 as compared to 3.8 in the keratinized stomachs and 6.2 in the stomachs with esophageal ulcers, hyperacidity was not associated with the gastric ulcer complex. In fact, these data indicate that the reverse may be true. The average hydrogen-ion concentration in esophageal region of normal stomachs was over 10 times

greater than the average hydrogen-ion concentration of stomachs with keratinization.

A possible relationship between the eosinophilic hepatitis complex and the gastric ulcer syndrome was not confirmed. On the basis of the epizoologic study, these 2 conditions appeared to be unrelated. In addition, other workers have not noted the eosinophilic hepatitis problem as being associated with the gastric ulcer complex.

Candida albicans was cultured from a high percentage of the keratinized lesions and ulcers in the esophageal region of the stomach. However, microscopically, the organisms were in the superficial layers and not in the submucosa. C. albicans was probably a secondary invader, but may have possibly interfered with healing. This is in agreement with the reports of numerous other research workers. In Experiment II, ulcers were not reproduced by oral administration of C. albicans. This also indicates that this organism represents a secondary factor in the gastric ulcer complex. Candida albicans, however, was capable of establishing itself by intravenous, subarachnoidal, and oral routes of administration. The information in this thesis indicates that C. albicans is pathogenic to pigs and causes a granulomatous type of inflammation.

In Experiment III, the use of the cortical steroid, prednisolone, indicated that stress factors play an important role in the gastric ulcer complex. Of the 12 pigs given prednisolone in 2 different trials for 7 days, 10 had esophageal ulcers in their stomachs; in contrast, 1 of 12 controls had an esophageal ulcer. Since the steroid-related stomach ulcers in pigs were not similar anatomically to the same type

in man and rats, this indicated a possible inherited weakness in the esophageal region to stress factors. Sections of the livers and hearts indicated extensive glycogen deposition which accounted for the increased weights of these organs in pigs given prednisolone. The decreased average weight of the stomachs of the prednisolone-treated pigs may have been due to its anti-inflammatory properties. Pigs fasted prior to slaughter usually have edema of the stomach wall, and perhaps prednisolone inhibited the edema that usually accompanies fasting. The blood values of the prednisolone-treated pigs showed a leukopenia with an absolute lymphopenia.

SUMMARY

The epizoology of the gastric ulcer problem was studied using 1071 slaughter-age pigs from the Swine Evaluation Station, Michigan State University Swine Farm, and outside sources. The study indicated 378 (35.3%) had keratinization of the stomach, 115 (10.7%) had ulcers in the esophageal region (hereinafter called esophageal ulcers), and 38 (3.5%) had fundic ulcers. The 170 pigs from the Swine Evaluation Station had the highest incidence of stomach lesions at slaughter. Although all the pigs were clinically normal, 39 of 115 with esophageal ulcers had free and clotted blood in their stomachs. The pigs subjected to the stresses associated with growth experiments and environmental and management changes had the highest incidence of stomach lesions. Keratinization of the esophageal region was characterized by a parakeratotic proliferation of the squamous epithelium. This appeared to precede necrosis and ulcer formation. The esophageal ulcers and fundic ulcers appeared as separate and unrelated entities.

No differences were noted in the incidence of stomach lesions in 97 pigs given rations varying in vitamin A and carotene content. The research on stomach pH values indicated that the average pH value was 2.5 in the normal stomachs, 3.3 in those with keratinization, and 6.2 in those with esophageal ulcers. Thus, low acidity, rather than hyperacidity, appeared to be related to the stomach lesions.

Candida albicans was frequently cultured from stomach lesions, but this organism was considered a secondary factor because ulcers could not be experimentally reproduced. Using 12 pigs, it was established that

C. albicans was capable of producing lesions following intravenous, subarachnoidal, and oral routes of administration.

Twenty-four pigs were used in the study of prednisolone-induced ulcers. Daily injections of prednisolone caused a high incidence of stomach ulcers, increased gluconeogenesis, inhibited inflammatory responses, and caused a leukopenia with an absolute lymphopenia.

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