RELATIONSHIP OF A SWINE PLEUROPNEUMONIA-LIKE ORGANISM TO INFECTIOUS ATROPHIC RHINITIS IN SWINE

by

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INTRODUCTION

Infectious atrophic rhinitis is a disease of swine characterized by progressive atrophy of the nasal turbinates. Its occurrence was not reported in the United States until 1944. Many members of the Veterinary Profession believe that it was present prior to this time, but no definite record of its occurrence is available. Since the recognition of this disease in the primary swine-producing area of the nation, there has been a great demand for information concerning it. It is surprising to many that more specific facts about this disease are not known. This paucity of knowledge results from two factors. One is that an understanding of its etiology has as yet eluded investigators and the second is that no laboratory animal has been found that is susceptible to this disease. A knowledge of the cause of infectious atrophic rhinitis can be expected to expedite the development of therapeutic and control measures. Therefore the present work has been directed towards the goal of determining the etiology of infectious atrophic rhinitis. Since no extensive review of the literature on this subject is available it appeared desirable to make the present review as complete as possible.

REVIEW OF LITERATURE

Infectious atrophic rhinitis has been recognized for a relatively long time in Germany. It was the German worker Franque (1830) who published the first report of this disease. He called the disease Schnüffelkrankheit (sniffling disease). His report was based upon information from two veterinarians who practiced in the mountainous region of Nassau in Germany. He recorded that these two veterinarians observed the affected swine did not fatten, developed an atrophy of the nasal turbinates and ethmoid turbinates with a subsequent malformation of the nose, and in severe cases exhibited nasal hemorrhage. Although the cause of this disease was not known it was speculated that short-nosed swine were more susceptible and that rachitic pigs rooting in stony ground might develop this condition. However Franque thought it more likely that the boar and sow hereditarily transmitted the condition to their offspring. It was observed that the disease spread gradually through an entire herd.

Although several articles on this disease were published during the next 75 years most of them presented basically similar ideas to those set forth by Franque with emphasis on heredity, nutrition, or an infectious process as the

cause. During this period the original name of Schnüffelkrankheit was used to signify an ever-increasing number of pathological conditions of the nasal cavity of swine. Jensen (1916) redefined the disease for which Franque had proposed this name, and separated the many conditions that were referred to by this term. With this brief background it will be easier to follow the literature of the period 1830-1916.

Hering (1842) included a short description of Schnüffelkrankheit in his special pathology text. This appears to be an abstract of Franque's original article. It is in the pathology textbook written by Spinola (1858) that the first description of several disease syndromes under the name of Schnüffelkrankheit appeared. He mentioned that the disease occurred as an acute rhinitis with marked swelling of the snout and in some cases the eyes even swelled shut. In addition, atrophy of the turbinate bones sometimes occurred as well as osteomalacia of the head bones and tuberculous lesions in the nasal cavity. He also mentioned that ascites and pleuritis were present in some chronic cases. He believed that the pigs did not develop the disease as the result of rooting in hard stony ground and suggested that it was an infectious disease.

Haubner (1873) felt that facial deformity resulting from rickets was the cause of Schnüffelkrankheit and that this disease could be cured in its early stage by the administration of bone salts and cod-liver oil. He cautioned that tuberculosis could cause some of the same lesions.

One of the earliest estimates of the length of time this disease had existed in Germany is found in an article by Schneider (1878) who was raised in the Nassau country of Germany and who had information from local residents that Schnüffelkrankheit had been recognized at least 70 to 80 years prior to the time of his writing. He reviewed the various theories concerning its etiology expressed by previous workers and concluded that pigs rooting in hard ground was not a factor in the production of this disease but that it was inherited. He observed atrophy of the turbinates in a diseased pig head he examined.

Schell (1890) described as Schnüffelkrankheit a case of osteosarcoma of the facial bones of a pig. Imminger (1890) likewise described as Schnüffelkrankheit an acute febrile rhinitis of swine. Besnoit (1903) felt that Schnüffelkrankheit of swine was due to a lime deficiency and that he could effect a cure in most cases by including lime phosphate in the ration fed the animal.

Koske (1906) isolated Bacillus pyocyaneus from the nasal cavity of young swine dying of acute rhinitis and

septicemia. He also isolated the same organism from the viscera of these swine. It is of interest that he reproduced acute rhinitis in his experimental pigs by the intranasal instillation of a culture of this organism. He mentioned that some workers referred to rickets, osteomalacia, actinomycosis or tuberculosis of the bone as Schnüffelkrankheit but that he felt that the term should be used to designate acute rhinitis due to Bacillus pyocyaneus.

According to Hintze (1909) Schnüffelkrankheit was related to osteodystrophia fibrosa of other domestic animals. Wirth (1910) examined a pig infected with Schnüffelkrankheit and likewise concluded that this was osteodystrophia fibrosa or deformans. Ingier (1913) examined a swine specimen affected with Schnüffelkrankheit and reported that active osteogenic tumor tissue was present in the facial lesions, in the body skeleton, and in the limb skeleton. Busolt (1912), after examination of preserved specimens from a pig affected with Schnüffelkrankheit, expressed the opinion that the condition resembled human rickets.

C. O. Jensen (1916) after reviewing the literature that had accumulated about snøvlesyge (infectious atrophic rhinitis, sneezing sickness, sniffling disease, Schnüffelkrankheit) pointed out that three distinct disease syndromes were included under one name. One of these was

acute infectious nasal catarrh; another was bone malformations such as osteopetrosis, osteomalacia, rickets or ostitis fibrosa deformans of the facial bones; the third condition was similar to the original disease described by Franque as Schnüffelkrankheit. Jensen mentioned that atrophy of the turbinates followed by chronic, purulent nasal catarrh was well known in Denmark and was referred to as snøvlesyge, nysesyge or snoftesyge. He felt that the term Schnüffelkrankheit or its Danish equivalent should be reserved for this specific disease syndrome. Jensen also noted that Bang had collected swine specimens in Denmark showing this disease syndrome as early as 1880. In 1916 a check of 4022 swine heads from a packing house revealed that 23 had definite turbinate atrophy. This disease was thought by Jensen to have the characteristics of an infectious disease and was reported to constitute a serious problem in certain swine-rearing areas of Denmark. In a limited experiment using two normal 6- to 8-week-old pigs in contact with two older, infected animals, no transmission was demonstrated. Jensen concluded that this disease might be due to an inherited defect. Jensen also reported that no similar disease had been observed among other domestic animals.

Jones (1952) believed he observed evidence of turbinate atrophy in pictures included in an article by Graham (1913).

However, Graham (1952) indicated that he was unable to definitely establish that the swine he worked with had atrophic rhinitis. Bennett (1953) mentioned Jones' observations.

A report of the successful treatment of infectious atrophic rhinitis was given by Petersen (1925). This treatment consisted of a course of potassium iodide medication in cases thought to be free of secondary pneumonia. Petersen (1926) mentioned that this disease was occasionally seen in very young pigs, and that in older pigs a central nervous system involvement might occur in association with the nasal catarrh. He concluded that it was most certainly a transmissible, enzootic infection. In some areas there was a variation in the extent of spread of the disease from year to year. He observed a marked variation in the virulence of this disease. It appeared to limit swine production on some farms while other farms had little trouble from it. Petersen further noted that the atrophic turbinates contained very little osseous tissue and that any damage to the nasal turbinates was permanent. He considered Lugol's solution to be a satisfactory treatment for infectious atrophic rhinitis and mentioned that his field collaborators also had success with this treatment.

Manninger (1930) suggested that the term ansteckender Nasenkatarrh (infectious nasal catarrh) was preferable to Schnüffelkrankheit. He then described this condition as an acute hemorrhagic rhinitis from which Poels had recovered Pasteurella sp.

Jensen (1933) postulated that infectious atrophic rhinitis was a true infectious disease. He mentioned that 5 to 10 per cent of the abattoir swine in Denmark showed some degree of turbinate damage. This article was abstracted by Biester (1935) and was the first information on infectious atrophic rhinitis to be published in the English language.

Eber and Meyn (1934) reported their observations in a herd of swine affected with acute rhinitis. They observed atrophy of the turbinate in one of the pigs. B. pyocyaneum was recovered from the acute rhinitis. While a culture of this organism instilled intranasally was without effect, they were able to reproduce the acute rhinitis with toxin from this culture when it was placed in the ethmoid turbinates through a trephined opening. They reported favorable results from immunizing this herd with B. pyocyaneum bacterin.

Zarickij (1934) believed that rhinitis of pigs in France and rhinoscleroma of man were due to similar bacteria.

He reported the recovery of B. friedlandin, presumed to be the cause of rhinoscleroma of man, from the nasal cavity of one pig he examined. While he used the term Schnüffelkrankheit for the swine condition he investigated, Zaricky's description was inadequate to establish it as infectious atrophic rhinitis.

Hoflund (1937a) stated that chronic atrophic rhinitis was fairly common in south and middle Sweden. He was unable to isolate any bacteria he could consider to be the cause of the condition. He also reported failure in transmitting the condition by placing normal and diseased swine together. Therefore he concluded that failure of the turbinates to develop was an inherited defect. He demonstrated that turbinate atrophy could be detected in the living animal by radiographs.

Thunberg (1937) took issue with the concept that this disease was an inherited defect and cited his experience of introducing the disease into a normal herd by means of an infected boar. Hoflund (1937b) replied to this criticism and cited additional support for his contention that the condition was a typical inherited defect.

Krage (1937) felt that the disease was not due to a specific dietary deficiency, bacteria, or virus but was due to a latent infection that manifested itself when an

inherited predisposing factor was present in the animal. He cited negative transmission trials to prove that some inherited predisposition was necessary.

One of the most significant and complete publications on this disease is that of Radtke (1938) who reported that Schnüffelkrankheit occurred only in association with Ferkelgrippe (piglet influenza). He found that the Riemser single-house method of raising swine used to control Ferkelgrippe also controlled Schnüffelkrankheit. These observations prompted him to conduct an extensive investigation of Schnüffelkrankheit.

In a very detailed study of the normal structure and development of the nasal cavity of swine he found that the ventral and dorsal turbinates, the ethmoid labyrinth and the maxillary sinuses are fully developed at birth. The first, second and third frontal sinuses and the sphenoid sinuses become macroscopically discernable after birth. The contours of the swine skull are altered by the pneumonization of the frontal sinuses which is not complete until about one and one-half years of age.

Radtke observed that the dorsal, ventral and ethmoid turbinates contain much cartilage at birth but that the dorsal and ventral turbinates are completely ossified by

the fourth week of life while the ethmoid turbinate is completely ossified by the eighth week of life.

Radtke's results indicated that the bacterial flora of the nasal cavity of healthy pigs was reasonably constant. He found that B. coli, diplococci, streptococci and micrococci were the most regularly occurring organisms. The numbers of bacteria decrease in the posterior portion of the nasal cavity. The ethmoid turbinates and the sinus cavities are usually sterile. From 60 to 70 per cent of the healthy swine nasal cavities he examined harbored Hemophilus suis.

In an attempt to establish the relationship of Ferkelgrippe to Schnüffelkrankheit he examined 52 Ferkelgrippe-free pigs 4 to 8 weeks of age and found no rhinitis. He then examined 104 Ferkelgrippe-positive pigs from 50 premises, and found 39 had definite rhinitis. The more advanced cases of rhinitis in this group had turbinate atrophy. In the examination of these specimens Radtke observed that short-nosed breeds of swine tended to have more severe lesions of Schnüffelkrankheit than long-nosed breeds.

Radtke found that intranasal inoculation of nasal exudate from Schnüffelkrankheit cases into susceptible pigs would produce bronchial pneumonia typical of Ferkelgrippe and that filtrates (Watt filter) of typical Ferkelgrippe bronchial pneumonia would produce nasal lesions

similar to the early lesions of Schnüffelkrankheit. He concluded that Schnüffelkrankheit was a localization of Ferkelgrippe in the upper respiratory tract. This is the first report of the successful experimental transmission of infectious atrophic rhinitis.

Thunberg and Carlström (1940) reported infectious atrophic rhinitis was associated with certain premises and that when litters from healthy sows were divided and a part of each litter placed in a healthy herd and a part in a diseased herd, the pigs in the diseased herd developed the disease while those in the normal herd did not. They felt there was no evidence of hereditary transmission and that mature sows exposed to the disease developed a transient infection and would not infect subsequent litters if the sows were farrowed in isolated quarters.

Böttcher (1941) suggested that swine from herds infected with Schnüffelkrankheit should not be used as breeding stock and that good sanitation would help control the disease. Reinboth (1940) concurred with Radtke's opinion that Schnüffelkrankheit was a localization of Ferkelgrippe in the upper respiratory tract of young pigs and that the control measures used to combat Ferkelgrippe would also control Schnüffelkrankheit.

A condition that may have been infectious atrophic rhinitis was reported in the United States by York (1941).

He described a herd condition characterized by persistent sneezing and nasal hemorrhage. According to Hancock (1952), York observed this disease in an Indiana swine herd.

Doyle, Donham and Hutchings (1944) reported the occurrence of a dystrophic or atrophic rhinitis in five herds they observed over a three-year period in Indiana. They mentioned that the disease had existed in one small area of Indiana for 20 to 25 years. In this small area it was considered to be of great economic importance, but it was not observed to be widely prevalent in the state. They felt that the condition they described was very similar to the European disease called chronic atrophic rhinitis, but they had no direct comparison to enable them to definitely state that the two conditions were the same. These workers felt that the disease they were describing and bull-nose were different.

Isa (1944) reported the occurrence of infectious atrophic rhinitis in pigs in Manitoba, Saskatchewan and Alberta, Canada. Apparently he had heard many vague reports about this disease for several years prior to this. He felt that it was not practical to attempt to distinguish between this disease and bull-nose so he discussed them as one even though he mentioned that many authorities separated the two conditions into infectious atrophic rhinitis and

bull-nose. He observed the chief economic importance of this condition to be retardation of growth of the pigs and the lowering of carcass grade. He stated that the infection could be transmitted through contaminated pens and runs, but it is not certain that he actually observed transmission of infectious atrophic rhinitis by means of contaminated pens.

Connell (1945) stated that a disease called bull-nose had been present in the prairie provinces of Canada for a number of years but was distinctly different from the condition produced by Actinomyces necrophorus. He described the symptoms of this peculiar type of bull-nose as frequent violent sneezing and distortion of the snout to the side or a shortening of the snout.

McClelland (1945) discussed an infectious rhinitis of swine which he also referred to as bull-nose. It is doubtful that his description was based entirely upon the condition we now refer to as infectious atrophic rhinitis.

Phillips (1946) stated that Kernkamp had observed infectious rhinitis in Minnesota. Phillips pointed out that the swine breeders of Ontario, Canada felt this was a comparatively new disease and had not been present in their area for much over three years. One of the first symptoms Phillips observed in infected swine was persistent

sneezing. This was sometimes followed by a distortion of the snout. Most of the infected animals had a black area below the eyes which was attributed to occlusion of the tear duct with resultant spilling of tears over the lower lid. This created a moist area that trapped dirt and dust. He found the macroscopic change that took place in the nasal cavity of pigs with turbinate atrophy consisted of a progressive dissolution of the softer bony structures of the nose. An early inflammation of the nasal mucosa was followed by decalcification of the turbinate bones. In some of the more severe cases only remnants of the turbinate mucosa were observed and these were completely devoid of bony structure. He postulated that a similar absorption of the harder facial bones produced the nasal distortion. The occurrence of an encephalitis in a small fraction of the infected pigs due to entrance of organisms through the damaged cribiform plate was observed. The majority of infectious atrophic rhinitis infected pigs did not gain as rapidly as normal pigs due to secondary bacterial infections.

Phillips was able to isolate Corynebacterium pyogenes from the nasal cavity in over 90 per cent of the cases he examined. He concluded that this organism was the most important secondary invader. A filtrate (type of filter not stated) prepared from nasal material from infected

swine produced typical lesions when instilled intranasally into 5-day-old pigs. Phillips felt the etiological agent was a virus with secondary bacterial infections influencing the severity of the lesion.

Slagsvold (1946) cited examples of the occurrence of an influenza-like disease of young pigs in association with infectious atrophic rhinitis. He observed indications of a common etiology for the two conditions. Infectious atrophic rhinitis was scheduled under Norwegian law in September, 1946.

Duthie (1947) failed to transmit infectious atrophic rhinitis by placing 6-week-old to 3-month-old infected pigs in contact with normal pigs of similar ages. He likewise failed to transmit the disease by instillation of a broth suspension of nasal exudate from infected pigs into the nasal cavity of 4-day-old to 3-month-old pigs.

Moyniahan (1947) attempted to produce the disease with bacterial cultures he recovered from the nasal cavity of infected pigs. Transmission of the disease with a crude nasal mucosa suspension was also tried. The inoculated pigs were 10 to 12 weeks of age. They remained normal.

Jones (1947a) reported infectious atrophic rhinitis could be so severe in some herds that it limited swine production. He observed that cats kept on infected premises often developed a purulent rhinitis and occasionally a

conjunctivitis. However 6-week-old kittens remained normal when inoculated intranasally with nasal mucosa suspensions from infected swine. Guinea pigs and rabbits were found to be refractive to infection with this disease. His most important finding was that 9-week-old pigs placed in contact with infected swine failed to develop the disease, but a sow and her litter of week-old pigs placed in contact with infected animals did develop the disease.

He found that bacteria-free filtrates prepared from nasal exudate of infected pigs failed to reproduce the disease when inoculated intranasally into baby pigs. It was noted that convalescent serum, Corynebacterium sp. hyperimmune serum or Corynebacterium sp. toxoid failed to alter the course of natural outbreaks of this disease. He suggested that a control program could be based upon the fact that sows, exposed to this disease after maturity, went through a mild attack of the disease and usually did not transmit it to subsequent litters when farrowed in isolated surroundings.

Jones (1947b) found that infectious atrophic rhinitis had not been recognized in Quebec at that time. He attributed some of the loss of condition in pigs affected with this disease to toxemia. While he recognized that all affected litters were potential carriers of infectious

atrophic rhinitis, he observed that if the brood sows in an infected herd showed no evidence of the disease, were moved to non-infected premises, and were farrowed in isolated lots, they would not transmit the disease to their pigs.

Sippel et al. (1947) reported the occurrence of infectious atrophic rhinitis in Georgia. Sandstedt (1948) noted that this disease appeared to be of an infectious nature but that it did not cause the unthriftiness in the infected pigs that swine pneumonia did. It interested him that infectious atrophic rhinitis was common in herds affected with swine pneumonia even though some of the animals failed to show lesions of both diseases. He investigated reports of infectious atrophic rhinitis occurring alone but always found it associated with swine pneumonia. He felt this suggested a common etiology for the two conditions.

Schofield (1948) reported the microscopic lesions of infectious atrophic rhinitis. He observed the initial reaction to be an infiltration of large lymphocytes into the stroma of the turbinate epithelium. The cells of the nasal epithelium appeared elongated or cuboidal and did not become stratified or squamous even in advanced cases. Later in the disease there was an increase in the number of

tubuloalveolar glands, accompanied by a mild proliferation of the fibrous tissue elements of the stroma. He observed that proliferation of the osteoblasts occurred even when the bone damage was scarcely perceptible, and in advanced cases the osteoblasts were present in enormous numbers. This was interpreted as indicating these cells were putting forth a heroic effort to rebuild the destroyed bone but they never succeeded in the task. He felt the disappearance of the bony plates of the turbinates was the most outstanding characteristic of this disease.

MacNabb (1948a) reported that his group had information indicating infectious atrophic rhinitis was a transmissible disease, but no bacteria had been isolated that could be considered as having etiological significance. Filtrates (type of filter not given) failed to reproduce the disease. In addition they noted no difference in the susceptibility of different breeds of swine to this disease. MacNabb (1948b) presented evidence to indicate no significant correlation existed between the length of the pig's nose and susceptibility to infectious atrophic rhinitis. In this work MacNabb reported the successful transmission of infectious atrophic rhinitis to pigs by a technique originated by McKay. This consisted of the inoculation of crude atrophic turbinate material subcutaneously into a rabbit.

Material collected from the resulting rabbit abscess produced turbinate atrophy when instilled into the nasal cavity of experimental pigs.

Bennett (1948) made the first definite diagnosis of infectious atrophic rhinitis in Iowa swine.

Duthie (1948) reported the occurrence of turbinate atrophy and distortion of the facial bones in a purebred Yorkshire sow he autopsied.

Phillips et al. (1948) demonstrated that it was possible to produce typical lesions of infectious atrophic rhinitis by the intranasal inoculation of baby pigs with crude infected turbinate suspensions. They also noted that bacterial cultures recovered from atrophic turbinates did not cause turbinate atrophy when inoculated intranasally into young pigs.

Gendreau (1948) exposed both inbred and non-inbred pigs to pigs infected with this disease. Since both groups developed typical lesions of the disease he concluded that inbreeding could not be considered the cause of the disease. He observed that 7- to 8-week-old pigs acquired infectious atrophic rhinitis when placed in a pen which had previously contained infected pigs. Cohrs (1949) discussed Schnüffelkrankheit on the basis of Radtke's findings, agreeing that it was merely a localization of Ferkelgrippe.

Gwatkin et al. (1949) mentioned that in their initial work they failed to transmit the disease when nasal washings from infected swine were instilled intranasally in 6- to 12-week-old pigs. However, they found that the inoculation of day-old pigs with nasal washings from infected pigs produced turbinate atrophy. Filtrates prepared by passing nasal washings from infected swine through a Seitz filter failed to produce any lesions when inoculated intranasally into day-old pigs. A coarse Mandler filter produced a filtrate that may have caused some turbinate atrophy in one pig. These workers also found they could freeze the nasal washings at -25° F., thaw them and produce turbinate atrophy with them. They attempted serum neutralization of nasal washings using serum from the same animal from which they collected the nasal washings. Their trial was not successful since the nasal washings used were not infectious. Infectious nasal material was inoculated into the yolk sac and into the chorioallantoic sac of chicken embryos. It was found that the fourth and sixth chicken embryo passage material was not infectious for pigs. No embryo lesions were described. Stains prepared from the nasal turbinate material failed to show any rickettsiae. The pH of the nasal washings they examined was found to range from 7.34 to 7.51. These workers observed the destructive process

seemed to have a predilection for the submucosa of the nasal turbinates.

Gilman (1949) cited McKay's unpublished experiments as indicating that Actinomyces necrophorus and Pasteurella multocida acted synergistically to produce turbinate atrophy. Gilman used rabbit abscess material produced by inoculation of crude atrophic turbinate material subcutaneously into a rabbit to initiate infectious atrophic rhinitis in his experimental pigs. The inoculated pigs developed turbinate atrophy. He found no indication that long-nosed swine were more resistant to this disease than short-nosed swine.

Gwatkin and Plummer (1949) attempted to find a laboratory animal susceptible to infectious atrophic rhinitis of swine. They instilled nasal washings and filtrates of nasal washings from infected pigs intranasally into mature mice, baby mice, rats, hamsters, guinea pigs and rabbits. They observed no lesions in the inoculated laboratory animals. They also injected infectious swine nasal washings close to the metatarsal bone of chicks and observed no apparent bone destruction.

Schofield and Jones (1950) reported atrophic rhinitis could be transmitted when nasal discharge from infected pigs was inoculated intranasally into recently farrowed pigs. These inoculated pigs readily transmitted the disease to

uninoculated litter mates. These workers found that instillation of feces and urine from infected pigs failed to transmit the infection. Their observations indicated the earliest gross changes of this disease were numerous small foci of congestion of the mucous membrane of the turbinate bones. In severe cases they observed the inorganic salts were almost entirely removed from the turbinate bone in from two to four weeks. In many early cases the external surface of the nasal turbinate was practically free of any inflammatory exudate but in more advanced cases a muco-purulent discharge was present. Histological examinations of diseased turbinates led these workers to conclude that the initial lesion consisted of scattered foci of degenerated and desquamated epithelial cells. In these areas cellular infiltration of the submucosa was observed. The infiltrating cells were mainly large lymphocytes that were not observed to extend beyond the outer layer of the periosteum even though the submucosa was densely packed with the cells. They suggested that the portal of entry for the infection was the ducts of the tubuloalveolar glands as evidenced by the accumulation of neutrophils at this site. Damage to the turbinate epithelium later became more extensive resulting in large denuded areas.

In more advanced cases an increase in the number of tubuloalveolar glands was observed. These were often

distended with mucus to the extent that cysts were formed. Even in the final stages of the disease the turbinate epithelial cells remained cuboidal or elongated and did not become stratified squamous epithelial cells as in primary atrophic rhinitis of man. One of the earliest changes observed by Schofield and Jones was proliferation of the osteoblasts. In the areas of proliferating osteoblasts there was frequently a rarefication of the bone. In advanced cases the osteoblasts were present in enormous numbers and filled the space left by the disappearing bone. They regarded this as indicating that these cells were attempting to rebuild the bone. The fibrous tissues elements of the stroma were observed to proliferate slowly, to cause an increase in density and to eventually surround both the arterioles and veins with a zone of dense fibrous tissue. These workers found that the organisms they recovered from the nasal cavity of infected swine were similar to those reported by other workers. They failed to consistently isolate any one particular species of bacteria. Their attempts to transmit the disease with filtrates (filter type not specified) gave negative results.

Doyle (1950) mentioned that pigs 10 weeks of age develop clinical evidence of the disease after their introduction into a herd infected with infectious atrophic

rhinitis. Ellison (1951) credits Twiehous with the statement that this disease had put 60 per cent of the Ontario pork producers out of business. Twiehous felt that the basic lesion in this disease resulted from a failure of the calcium salts to precipitate out in the osteoid tissue. Some of the infected pigs he observed had an increase in the length of the snout. An editorial appearing in the Jen-Sal Journal (1951) states that this disease is one of the most serious diseases of swine, and is present throughout most sections of the United States.

Gwatkin et al. (1951) reported additional studies on the etiology of infectious atrophic rhinitis. They observed that anemia had no effect on the development of experimental cases of the disease, and that rabbit-abscess material produced by the subcutaneous inoculation of crude atrophic turbinate suspension did not produce turbinate atrophy in young pigs. In addition it was found that aerobic and anaerobic cultures of some of the most commonly encountered bacteria in the diseased swine nasal cavities did not produce turbinate lesions when instilled intranasally into young pigs. Filtrates prepared from infective material (Seitz and Mandler filters) proved to be noninfective for young pigs. They found that crude atrophic turbinate suspensions heated at 45° C. for 1 hour still produced the

disease although heating at 65° C. for 1 hour destroyed the infectivity of the material. Approximately 2400 mcg. of streptomycin and 3300 IU of penicillin per ml. added to the inoculum 30 minutes prior to inoculation prevented the development of lesions in the inoculated pigs. They observed that a combination of supernate and washed sediment obtained by centrifugation at from 12,000 to 20,000 R.P.M. produced a higher incidence of lesions than either material alone.

Switzer (1951) was the first to report the occurrence of Trichomonas sp. in the nasal cavity of swine with infectious atrophic rhinitis. He found 70 of 87 (80 per cent) Iowa swine affected with infectious atrophic rhinitis harbored this protozoon in their nasal cavities while only 2 of 72 (2.8 per cent) swine with grossly normal nasal cavities had this protozoon present. This protozoon was grown in bacteria-free and yeast-free cultures. It failed to become established when instilled into the nasal cavities of normal baby pigs. He did succeed in establishing the swine nasal trichomonad and Trichomonas suis in the bovine vagina.

Bennett (1951) reported that of the pigs over three weeks of age submitted to the Iowa Veterinary Medical Diagnostic Laboratory during a 6-week period, 59 had

gross lesions of infectious atrophic rhinitis while 83 showed no gross evidence of it. This indicated an incidence of 41.5 per cent.

Hutchings (1951) postulated that a virus might be the cause of infectious atrophic rhinitis. It was his impression that the case history of this disease was unique since it usually required three years for the disease to build up in a herd to the point it could be recognized. He noted that the only method of controlling this disease was complete depopulation of the swine drove, disinfection of the premises and restocking a few months later with pigs free of the disease.

Montgomery (1952) mentioned that some herds had sent as many as 800 pigs to the rendering plant due to this disease. He stressed the fact that infectious atrophic rhinitis could put swine producers out of business.

Spear (1952a) wrote of the danger to the swine industry occasioned by this disease. He stated that the condition was rapidly spreading in Iowa and that if it was not checked it could work havoc on the swine industry.

Kernkamp (1952) observed that pigs affected with infectious atrophic rhinitis sometimes took twice as much feed per pound of gain as normal pigs. He noted that pigs with this disease frequently had bronchial pneumonia, but he

cautioned that pulmonary and gastro-intestinal disturbances were not especially characteristic of this disease.

Jones (1952) found that Corynebacterium pyogenes antiserum and bacto-toxoid prepared from this organism failed to control a natural outbreak of infectious atrophic rhinitis. Likewise Pasteurella multocida antiserum and bacterin were of no value in controlling this infection. However, an intramuscular injection of 10,000 mcg. of streptomycin given three times during the first month after birth appeared to reduce the incidence of the disease but did not completely control it.

Spear (1952b) observed that the majority of infected pigs became rough in appearance and failed to make satisfactory gains. He reported that some herd owners controlled and eliminated this infection by early recognition and destruction of infected animals.

Simms (1952) reported that workers in the Bureau of Animal Industry had observed nasal trichomonads were often associated with infectious atrophic rhinitis. He suggested that these organisms were probably involved in one way or another in the production of this disease. Gwatkin and Dzenis (1952) found that 100 mg. of streptomycin administered intranasally seven days after the first inoculation appeared to protect 10 out of 12 pigs inoculated intranasally with infectious atrophic rhinitis material.

Messmore (1952a) stated that infectious atrophic rhinitis was a manifestation of swine erysipelas. Laboratory workers isolated cultures of E. rhusiopathiae from the nasal mucosa of swine specimens he submitted to them. This finding in addition to clinical observations caused Messmore to conclude that this bacterium was the cause of infectious atrophic rhinitis. Messmore (1952b) reiterated that he had never seen cases of clinical infectious atrophic rhinitis in a herd where some form of swine erysipelas could not be demonstrated. He employed penicillin and swine erysipelas antiserum for the treatment of infectious atrophic rhinitis.

Gray (1952) believed that infectious atrophic rhinitis existed throughout the Corn Belt and in all areas where there was a concentration of swine. He noted that a drove of swine could be infected with this disease and show no symptoms indicative of the infection. He stressed the fact that this disease built up in herds of swine, reaching its peak about the third year.

An editorial in the Farm Journal (1953) announced the merits of the otoscope for the detection of turbinate damage. The editorial cautioned that it was still too early to be certain of the role of the otoscope in the diagnosis of this disease.

Earl and Shuman (1953) found the otoscope could be used to determine the degree of turbinate destruction present in infected pigs. They believed this method offered a practical means to diagnose infectious atrophic rhinitis in individual animals.

Shuman et al. (1953) reported observations made on a herd with infectious atrophic rhinitis. They found that 60 per cent of the pigs with turbinate atrophy had pneumonia while 50 per cent of the pigs with grossly normal turbinates also had pneumonic lungs. They obtained no evidence that any specific bacteria caused this disease, or that swine influenza virus was concerned in the production of either this disease or the swine pneumonia they observed.

Shuman and Earl (1953) attempted to evaluate the accuracy of rhinoscopic detection of turbinate damage. They could detect by rhinoscopic examination about 75 per cent of the cases that exhibited turbinate atrophy at necropsy.

Aitken (1953) suggested that a course of treatment with sulfapyridine was a diagnostic aid to differentiate infectious atrophic rhinitis from bull-nose of swine. If the pigs recovered from the disease after this treatment it was assumed they had bull-nose.

Dykstra (1953) reported that he knew of no such condition as a mild case of infectious atrophic rhinitis. It

was his belief that this disease always produced complete destruction of the turbinate bones if allowed to progress. He did not believe an immunity developed in this disease. A period of two months was recommended as sufficient time to eliminate the infection from swine-free premises. Smiley (1953) presented a detailed account of this disease building up in a pure-bred herd and becoming disseminated to other herds through the sale of breeding stock.

Borgmann (1953) demonstrated that Erysipelothrix rhusiopathiae was not the cause of infectious atrophic rhinitis and did not appear to have a synergistic action in the production of this disease. An editorial appearing in Veterinary Medicine (1953) cautioned that failure to demonstrate trichomonads in the nasal cavity of swine with infectious atrophic rhinitis did not always indicate they were not present.

Shuman (1953) indicated that a difference in rate of infection was noted between pigs kept on concrete and those put out on pasture, the latter group having an unexpectedly high incidence of infectious atrophic rhinitis. Spindler et al. (1953) considered their experimental results established an etiological relationship between trichomonads and infectious atrophic rhinitis of swine. Their evidence indicated the trichomonads might be of either nasal or intestinal origin and still produce turbinate atrophy.

An editorial appearing in the Fort Dodge Bio-Chemic Review (1953) stated that clinical evidence of infectious atrophic rhinitis in a few animals of a group offered for sale at a public sales barn should warrant condemnation of the entire group.

Simms (1953) reported that workers in the Bureau of Animal Industry examined several hundred stillborn pigs or pigs dying at a few days of age, and failed to find any evidence of turbinate damage regardless of whether the dam had infectious atrophic rhinitis or was normal. He also reported that there was no significant difference in weight gain between pigs with normal turbinates and those with atrophic turbinates in the two herds they studied. Of a group of pigs on pasture 67.3 per cent were affected with this disease while only 27.4 per cent of a similar group of pigs on concrete showed turbinate lesions.

In Veterinary Excerpts (1953) the conclusion was reached that the evidence strongly indicated trichomonad infection was the cause of infectious atrophic rhinitis. Ray (1953) believed that trichomonads were common secondary invaders in cases of infectious atrophic rhinitis simply because the accumulated pus served as a good culture medium for them.

Goldstein (1953) concluded there was insufficient evidence to establish trichomonads as the etiology of infectious

atrophic rhinitis. He also believed that in some cases of the disease pneumonia entirely accounted for the unthriftiness.

Gwatkin et al. (1953) found that Pasteurella multocida was present in the nasal cavities of 38 per cent of the swine with atrophic turbinates they examined and in only 16 per cent of the swine with normal nasal cavities. Consequently they inoculated a culture of Pasteurella multocida recovered from a diseased nasal cavity, into the nasal cavities of two susceptible pigs. One of these pigs had turbinate atrophy when necropsied two months later. When the culture was combined with a filtrate (Seitz filter) of atrophic turbinate before inoculation, two out of three inoculated pigs developed turbinate atrophy. When the Pasteurella multocida culture was inoculated intranasally into rabbits most of them died but one lived for two weeks and had turbinate atrophy when necropsied.

Switzer (1953a) reported the isolation of a filterable agent from the nasal cavity of swine with infectious atrophic rhinitis. He propagated this agent in embryonated chicken eggs and observed that it appeared to be a pleuropneumonia-like organism. The outstanding lesion observed in the inoculated chicken embryos was severe pericarditis. He observed that a filtrate of a crude

atrophic turbinate suspension (Selas number 02 filter) produced mild turbinate changes in five young pigs.

McKay and Carter (1953a) observed L forms of Spherophorus necrophorus in 71 cases of infectious atrophic rhinitis, however they did not observe the tissue necrosis usually associated with S. necrophorus infections. The reversion of these L forms to bacillary forms on prolonged incubation and repeated subculture was their basis for considering them as derived from S. necrophorus.

McKay and Carter (1953b) reported that turbinate atrophy in swine could be produced by the instillation of rabbit abscess material produced by the injection of crude atrophic swine turbinate material subcutaneously into rabbits. After one or two rabbit passages the rabbit abscess material consistently yielded Pasteurella multocida and L-type colonies of Spherophorus necrophorus. However pure cultures of P. multocida failed to produce turbinate atrophy in any of the pigs they inoculated.

Switzer (1953b) found that when a suspected pleuropneumonia-like organism he had isolated from the nasal mucosa of swine with atrophic turbinates was instilled intraperitoneally into pigs six weeks or less of age severe fibrinous pericarditis, pleuritis and peritonitis resulted. Since the lesions in the inoculated pigs

resembled those in field swine with pericarditis, peritonitis and pleuritis, several such field cases were examined for the presence of this suspected pleuropneumonia-like agent. He found eight of nine of the field cases of pericarditis, peritonitis or pleuritis positive for the suspected pleuropneumonia-like organism. However, when he instilled this agent into the nasal cavities of 14 baby pigs, no gross atrophy of the nasal turbinates resulted even though the agent became established in the nasal cavity. These inoculated pigs had a mild infiltration of the submucosa of the nasal turbinate with lymphocytes, lymphoblasts and macrophages.

Myers (1953a, 1953b and 1953c) believed that infectious atrophic rhinitis was caused by a nutritional deficiency with secondary bacterial invaders producing the turbinate atrophy.

Carter and McKay (1953) reported that many of the organisms referred to in their previous reports, McKay and Carter (1953a and 1953b), as L-type colonies of S. necrophorus were in reality pleuropneumonia-like organisms since many of them did not revert to a bacillary phase or show any evidence of gas production. Serum enriched thioglycollate broth was found to be a satisfactory medium for the cultivation of the swine pleuropneumonia-like

organism. They observed colony differences between the L-type colonies of S. necrophorus and the pleuropneumonia-like organism present in swine. They confirmed Switzer's (1953a) observation that a Selas number 02 filter would allow the pleuropneumonia-like organisms to pass. They published their observations in order that the earlier reports of McKay and Carter (1953a and 1953b) might be more readily oriented with those of Switzer (1953a and 1953b).

Phillips (1953) expressed the opinion that a synergistic relationship between two agents might still prove to be the cause of infectious atrophic rhinitis.

Smith (1953) found that pigs four to eight weeks of age placed in contact with field cases of infectious atrophic rhinitis did not develop lesions of the disease. He felt that the otoscope was an aid in diagnosing this disease.

Gwatkin et al. (1953) found that a pure culture of Pasteurella multocida instilled intranasally into young pigs produced nasal changes typical of infectious atrophic rhinitis. They also inoculated a culture of Pasteurella multocida isolated from the nasal cavity of an infected pig, into the nasal cavity of rabbits where it appeared to produce turbinate atrophy in the surviving animals. Gwatkin and Dzenis (1953) reported additional trials using this

culture of Pasteurella multocida. It appeared to produce typical lesions of infectious atrophic rhinitis when inoculated into young pigs. They isolated cultures of Pasteurella multocida from six field cases of infectious atrophic rhinitis and produced typical lesions in experimental pigs by the intranasal inoculation of these isolates. In addition a Pasteurella multocida culture isolated from the lung of a pig with acute pasteurellosis produced typical turbinate atrophy when inoculated intranasally into young pigs.

Schofield and Robertson (1953) observed turbinate atrophy in baby pigs inoculated with atrophic turbinate material containing no demonstrable trichomonads. In addition they found that some rabbit abscess material produced by the subcutaneous inoculation of atrophic turbinate suspension, caused turbinate changes in inoculated pigs but some lots of rabbit abscess material did not. They observed that instillation of total bacterial cultures, namely, a composite of the bacteria washed from the surface of culture plates inoculated with atrophic turbinate material and incubated aerobically, anaerobically and under increased carbon dioxide atmosphere, would produce turbinate atrophy in some of the inoculate pigs. No pen transmission occurred when pens that had contained infectious atrophic rhinitis

infected pigs were left idle for three weeks before young susceptible pigs were placed in them. In the discussion following their paper they mentioned that the turbinate damage was most likely due to injury to the osteoblasts.

Flatla and Braend (1953) found that hereditary factors were not important in the etiology of this disease. They initially believed Hemophilus sp. might have etiological importance in this disease but later found it would not produce turbinate atrophy. However cultures of Pasteurella multocida instilled intranasally produced typical turbinate atrophy. They found that detection of turbinate atrophy by radiographs assisted them in sorting out infected animals.

An editorial in The Veterinary Record (1954a) contained a notice issued by the Ministry of Agriculture and Fisheries on May 20, 1954. This notice reported that for the first time on record, the existence of atrophic rhinitis had been confirmed among pigs in Great Britain. This notice stated that little doubt existed that the infection had been introduced by an apparently healthy Landrace pig imported into England from Sweden. An editorial appearing in The British Veterinary Journal (1954) expressed gratitude for the recent atrophic rhinitis order in Great Britain since it indicated that the officials responsible for animal protection were alert to recognize this new disease.

A second editorial appeared in The Veterinary Record (1954b) and quoted the Atrophic Rhinitis Order in full. This order provided for the quarantine of infected and contact pigs, their destruction if deemed necessary, and the disinfection of the contaminated premises.

Switzer (1954) reported recovery of his previously described suspected pleuropneumonia-like organism from 20 of 28 pneumonic swine lungs. This agent was recovered from the nasal cavities of 14 of the 20 animals with positive lungs. This suggested a correlation between the occurrence of this agent in the nasal cavity and in the pneumonic lung.

Levine et al. (1954) carried out an investigation to determine the relationship of trichomonads to atrophic rhinitis. These workers observed that 10 of 11 pigs with turbinate atrophy had nasal trichomonads present while 9 of 23 pigs with no nasal lesions also harbored this protozoon. They were unable to produce turbinate lesions in 11- to 39-day-old pigs by the intranasal inoculation of bacteria-free cultures of nasal trichomonads recovered from field cases of infectious atrophic rhinitis.

Carter (1954) found that baby pigs inoculated intranasally with cultures of the swine pleuropneumonia-like organism did not develop turbinate atrophy. He isolated this same organism from three different field outbreaks of

serofibrinous pericarditis, pleuritis and peritonitis in swine. Young pigs inoculated intraperitoneally with cultures of this organism developed lesions similar to those observed in the field cases. The organism was recovered from the lesions in the experimental pigs. He failed to note the similarity between his findings and those reported by Switzer (1953b) even though Switzer's work is included in his list of references.

Heddleston et al. (1954) reported isolation of Pasteurella multocida from the nasal cavities of 6 of 76 (8.0 per cent) swine with infectious atrophic rhinitis and from the nasal cavities of 4 of 96 (4.3 per cent) rhinitis negative swine.

Switzer (1954b) reported on current information concerning the etiology and control of infectious atrophic rhinitis. He presented evidence indicating that Spherophorus necrophorus, Pasteurella multocida and nasal trichomonads were not concerned in the production of turbinate atrophy in an isolated herd of infected swine. Selas number 02 filters did not allow the atrophy producing agent to pass. In a very limited survey he found one swine pneumonia specimen that yielded no bacterial growth on aerobic blood agar cultures but which produced a very definite turbinate atrophy when inoculated intranasally into

baby pigs. This specimen was from a pig with severe turbinate atrophy. He reported the cultivation in artificial medium of the filterable agent he had previously isolated. This was found to be a typical pleuropneumonia-like organism. He outlined three general control plans. The plan that appeared to be the most effective was to take the pigs from the dam when they were 24 hours or less of age, and to rear them in isolation.

Braend and Flatla (1954) reported the details of the research work they had discussed in a previous paper (Flatla and Braend (1953)). They found this disease was not an inherited defect but was apparently due to a bacterial infection. They reported that Pasteurella multocida would produce typical turbinate atrophy in the inoculated pigs. The technique of Hoflund (1937a) of demonstrating turbinate atrophy by radiographs was amplified. This technique allowed detection of somewhat less than seventy-five per cent of the infected swine.

These workers suggested that the most effective method of controlling this disease is complete depopulation of the swine on an infected farm followed by restocking with normal swine. Another method they felt could be used to eradicate the disease was isolation of bred sows. These sows were farrowed in individual, isolated houses and

the litters were kept in isolation for five months. Breeding stock was selected at the end of this time from the litters that appeared normal. These workers also reported that local and systemic treatment of the sows with streptomycin was an aid in eliminating the infection but they cautioned that the use of antibiotics in this disease might result in resistant strains of organisms that would be very difficult to control.

OBJECTIVE

Within two years after the initial cases of infectious atrophic rhinitis were diagnosed in Iowa, it became apparent that this disease was relatively widespread. As the Veterinary Profession was faced with the problem of controlling the spread of this disease and the treatment of infected herds, it became evident that little specific information about this disease was available. The intervening time has not materially advanced our knowledge of its etiology. Several workers have reported that the disease is transmissible but there is considerable disagreement as to the nature of the etiological agent. The literature contained two reports that filtrates prepared from nasal washings collected from swine with infectious atrophic rhinitis produced turbinate lesions similar to the natural disease when inoculated into the nasal cavity of normal pigs. On the other hand numerous workers have reported that they obtained no evidence of the transmission of this disease by filtrates. In addition to these conflicting reports several investigators have found indications of a common etiology between infectious atrophic rhinitis and an influenza-like disease of swine.

These stimulating observations and conflicting results indicated there was need for a critical study of the possible existence in the nasal cavity of swine of unknown filterable agents. Therefore this work was undertaken in an attempt to advance our knowledge of this disease, especially in regard to its etiology.

It was possible in this current work to isolate a pleuropneumonia-like organism from the nasal cavity of swine. This was the first time that such an organism had been reported from swine. It has been briefly discussed in four published papers (Switzer 1953a, 1953b, 1954a and 1954b). It is the purpose of the present work to further elaborate on this organism and to establish the relationship of the swine pleuropneumonia-like organism to infectious atrophic rhinitis.

METHODS OF PROCEDURE

Source of Infectious Atrophic Rhinitis Material

It was apparent at the onset of this study that a herd of swine affected with infectious atrophic rhinitis would have to be maintained in order that similar infectious material could be used in different experiments. A small infected herd was already established at the Veterinary Medical Research Institute when work on this problem was started. The infection in this herd was maintained by serial passage of crude atrophic turbinate material inoculated intranasally into susceptible baby pigs. When the infected pigs reached three or four months of age one or more of them were necropsied and atrophic turbinate material was collected for inoculation into the next litter of baby pigs. The inoculated baby pigs developed severe turbinate atrophy during the first few serial passages but at the end of two years the virulence of the infection had decreased enough that severe turbinate atrophy occurred only in an occasional litter. This herd was maintained on a ration containing no antibiotics. They were housed in individual pens in a central farrowing house. On pasture

they were maintained in small groups. These swine were kept under better than average sanitary conditions.

In addition, specimens were obtained from field cases of infectious atrophic rhinitis through the cooperation of the Iowa Veterinary Medical Research Institute's field collaborator and through the Iowa Veterinary Medical Diagnostic Laboratory.

Preparation of Filtrates

Filtrates of nasal turbinates were prepared from more than 185 specimens. It was found that satisfactory filtrates could be prepared by the following technique. The turbinate was dissected out of the nasal cavity with sterile scissors and ground in a sterile mortar with alundum. Enough sterile tryptose broth was added to the ground tissue to make a 1 to 2 per cent tissue suspension. This suspension was centrifuged at 2,000 R.P.M. for 15 minutes. The supernatant fluid was clarified by filtration through a Selas number 10 filter. This material was then filtered through a Selas number 02 filter at a negative pressure of from 8 to 10 inches of Hg.

A Selas number 02 filter was used in the initial isolation of this agent. When attempts were made to filter the

swine pleuropneumonia-like organism through Seitz S.T. 8 filter pads or Mandler filter candles of 7 to 10 pounds bubbling pressure the filtrate did not contain detectable pleuropneumonia-like organisms. Therefore the Selas number 02 filter was routinely used in this study.

Cultivation of the Swine Pleuropneumonia-like Organism in Chicken Embryos

When material containing the swine pleuropneumonia-like organism was inoculated into 7-day-old chicken embryos by way of the yolk sac, amnioallantoic sac, or on the chorioallantoic membrane it was observed that the yolk sac route of inoculation usually resulted in more severe lesions and in a higher mortality. Therefore the yolk sac route of inoculation was routinely employed in this work. Since many of the surviving chicken embryos had lesions when necropsied 10 days postinoculation, typical lesions were considered to indicate infection of the chicken embryo, as was death of the chicken embryo. All chicken embryos were incubated at 37° C. after inoculation. The chicken embryos were usually 7 or 8 days old when inoculated.

Titration trials were conducted using tenfold dilutions with tryptose broth employed as the diluent. Two-tenths ml. of each dilution was inoculated into twelve 7-day-old chicken embryos by way of the yolk sac. The ID_{50} of each series was calculated according to the method of Reed and Muench (1938). All chicken embryos used in this work were obtained from an isolated flock maintained at the Veterinary Medical Research Institute. This flock showed no evidence of chronic respiratory disease.

Cultivation of the Swine Pleuropneumonia-like Organism in Artificial Medium

The swine pleuropneumonia-like organism was grown in fresh ox heart infusion to which was added hemoglobin and chicken serum. The medium was prepared by passing fresh ox hearts with the fat trimmed off through a meat grinder and allowing 1 part ground heart to infuse in 2 parts distilled water for 12 hours at approximately 5° C. The infusion was then heated in a boiling water bath for 30 minutes and filtered through coarse filter paper. The infusion was adjusted to a pH of 7.8 with 0.10 N NaOH after which 0.2 per cent Bacto-hemoglobin and 0.5 per cent NaCl

were added and the infusion again heated in a boiling water bath for 30 minutes. When the medium had cooled it was filtered through filter paper and a Seitz clarifying pad. At this time 20 per cent heat inactivated chicken serum was added and the complete medium sterilized by filtration through a Selas number 03 filter candle. It was dispensed into sterile screw-top tubes 13 mm. x 125 mm. in 7 to 8 ml. amounts. The sterility was checked by incubation of the tubed medium at 37° C. for 24 hours. After this it was stored at 5° C. until needed.

When it was desired to isolate cultures from contaminated material, thallous acetate to make a final concentration of 1-4,000 and 10,000 to 20,000 units of crystalline penicillin per ml. were added to the medium. Visible growth of the swine pleuropneumonia-like organism usually occurred in 24 to 48 hours. Growth of the organism imparted a slight turbidity to the medium. It was essential that uninoculated control tubes be included in each transfer for comparison purposes. Transfers were made at 48-hour or 72-hour intervals. Cultures transferred after 7 days incubation were usually non-viable. Growth of the pleuropneumonia-like organism was routinely verified by examination of smears prepared from the sediment in the culture tubes after centrifugation at 2500 R.P.M. for 15 minutes. These were stained with Giemsa's stain.

Pig Inoculations

When the initial pig inoculations were made it was believed that an isolated breeding herd free of infectious atrophic rhinitis was available as a source of experimental pigs. Therefore, sows from this herd were brought into individual isolation units where they farrowed. The pigs remained with their dams during the course of the experiment. After a few trials of this nature an outbreak of pneumonia and infectious atrophic rhinitis was observed in one lot of weaned pigs in the breeding herd. This led to a more critical appraisal of this herd. It was decided to take experimental litters from the dams when the pigs were 24 hours or less of age and to rear them by hand in isolation units. Some of the pigs from each litter were placed in a separate isolation unit to serve as uninoculated control pigs. More than 30 litters were raised by this method and none of the uninoculated control pigs showed evidence of infectious atrophic rhinitis.

The ration fed to the baby pigs consisted of fresh concentrated milk obtained from the Iowa State College Department of Dairy Industry, diluted with an equal amount of water. Vitamins A and D were added to the milk. In addition 5 ml. of the mineral mixture described by Young

(1951) was added to each quart of milk. The pigs were fed in flat pans and fountains. They were fed three times a day for the first 10 days and twice daily thereafter. At no time were antibiotics fed to the pigs. A trough of the ground pig feed used to maintain the Veterinary Medical Research Institute swine herd was placed in the pen on the second day. The milk portion of the ration was discontinued after the fourth week. Each pig was initially fed 100 ml. of milk at each feeding. This was gradually increased to 250 ml. It was found advantageous to keep the pigs slightly hungry since diarrhea occurred less frequently than when the pigs were given all the milk they would drink. It was possible to arrange the work of the caretaker so that he contacted only the pigs involved in the experiments.

Histological Technique

Satisfactory tissue sections of the nasal turbinates were obtained by the following technique. The nasal turbinates were fixed in Zenker's fixative plus 2 to 3 per cent glacial acetic acid at approximately 5° C. This decalcified the tissues in 24 hours. The tissues were washed in running tap water for 24 hours; dehydrated by successive changes of

70 per cent, 90 per cent and 100 per cent ethyl alcohol; transferred to dioxane; and embedded in paraffin at 56° C. The tissues were then sectioned, attached to clean glass slides and dried. After this the tissue sections were transferred to xylene followed by successive transfers to 100 per cent, 90 per cent and 70 per cent ethyl alcohol and then to tap water. They were then transferred to dilute Lugol's solution and then to tap water. The tissue sections were stained in a 1:50 aqueous solution of Giemsa's stain for one to two hours. They were destained to the desired density in ethyl alcohol to which was added 6 drops of glacial acetic acid per 50 ml. of alcohol. Electric decalcification of the tissues was tried but did not result in as satisfactory preparations as the previously described Zenker's fixative-acetic acid method. Haematoxylin-eosin stained tissue sections were not as satisfactory as those stained with Giemsa's stain.

To prepare smears of the swine pleuropneumonia-like organism, the cultures were concentrated by centrifugation at 2,000 R.P.M. for 15 minutes, the sediment was spread on clean glass slides, air-dried, warmed over a flame, fixed in absolute methyl alcohol for 2 minutes, air-dried, and then stained with standard Giemsa's stain for 1 hour and 15 minutes.

Gross Examination of Nasal Turbinates

The determination of turbinate atrophy was accomplished by visual inspection. In order to do this the nasal cavity was usually cut at right angles to the longitudinal axis immediately anterior to the first premolar tooth. In some cases the nasal cavity was cut along the longitudinal axis since the split nasal septum could be dissected out and specimens collected from the nasal turbinates with no extraneous contamination. In most cases the turbinate atrophy was obvious. However there were a few cases in which the changes were so slight that interpretation was difficult.

Preparation of Material for Electron Microscopy

The swine pleuropneumonia-like organisms examined with the aid of the electron microscope were from a 48-hour-old culture grown in ox heart infusion-chicken serum medium. The organisms were concentrated by centrifugation at 2,500 R.P.M. for 15 minutes. The sediment was resuspended in a volume of sterile distilled water equal to the original culture. The organisms were again centrifuged using the

same procedure as before. A portion of the sediment was transferred to approximately 50 times its volume of sterile distilled water. About 0.03 ml. of this suspension was placed on a previously prepared collodion membrane supported by a 200 mesh screen grid and allowed to dry. The preparations were shadow cast with gold at an angle of approximately 20°.

The collodion membranes were prepared by floating one drop of a mixture of 1 part U.S.P. collodion and 4 parts n-amyl acetate on the surface of water contained in a glass dish approximately 10 inches in diameter. The screen grids were placed on this film and the screen grids plus the membrane were removed by scooping them up on a clean glass slide in such a manner that the screen grid was next to the glass slide and was covered by the membrane.

Examination of Atrophic Turbinates for the Presence of *Pasteurella multocida* and *Spherophorus necrophorus*

The atrophic turbinates were exposed by cutting through the head along the longitudinal axis and dissecting out the nasal septum. Exudate was collected from the middle one-third of the ventral nasal turbinate and streaked on blood agar plates which were then incubated aerobically at 37.5° C.

These cultures were examined at 24 hours and 48 hours. Any colonies resembling Pasteurella multocida were transferred to tryptose broth. The fermentation reactions in lactose, sucrose, dextrose, maltose, and mannite enriched media were determined. The action of the culture on litmus milk was ascertained. The presence or absence of indol and hydrogen sulfide production was determined in Difco S.I.M. medium.

The presence or absence of Spherophorus necrophorus was demonstrated by the inoculation of 1 ml. of a 1 to 2 per cent tissue suspension of atrophic turbinate material subcutaneously into a mature white rabbit. The rabbit was necropsied five days after inoculation and examined for subcutaneous abscesses or necrosis.

RESULTS

Lesions of Infectious Atrophic Rhinitis

The early gross changes in this disease are a slight accumulation of mucous exudate on the epithelium of the turbinates and an atrophy of some portion of the turbinates. It was found that the inferior scroll of the ventral turbinate was almost always the area that showed the first discernable atrophy. In some cases there was a severe atrophy of the inferior scroll while no other portion of the turbinates exhibit any gross atrophy. However, in a few cases the ethmoid turbinates or the superior scroll of the ventral turbinates were atrophic while the inferior scroll was normal in appearance. Figures 1 and 2 illustrate a normal pig. Figures 3, 4, 5 and 6 illustrate severe cases of infectious atrophic rhinitis.

No inflammation of the nasal mucosa was observed in uncomplicated cases of this disease. In fact, many of the infected animals had a decrease in the vascularity of the nasal mucosa as compared to the normal, uninoculated control animals. Many of the swine with turbinate atrophy also had some degree of pneumonia, but some of the inoculated

Figure 1. Dorsal view of the head of a normal 8-week-old pig.

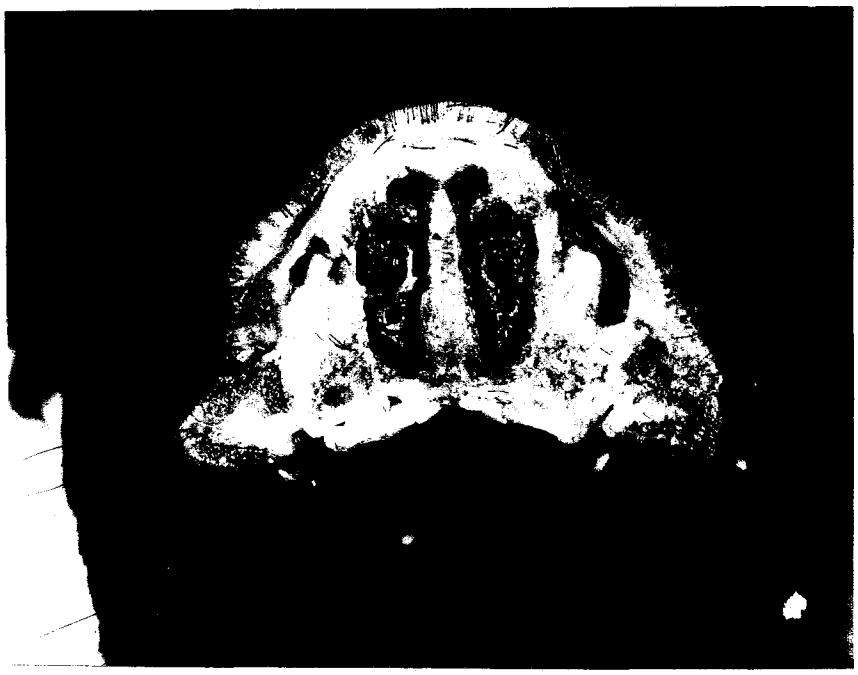

Figure 2. The same pig head as Figure 1 but sectioned to show the normal nasal turbinates.

Figure 3. A pig with infectious atrophic rhinitis. The nose is shortened and there is excessive wrinkling of the skin just back of the snout.

Figure 4. The same pig head as Figure 3 but sectioned to show the severe atrophy of both nasal turbinates.

Figure 5. Dorsal view of the head of a pig with infectious atrophic rhinitis. Note the lateral distortion of the nose.

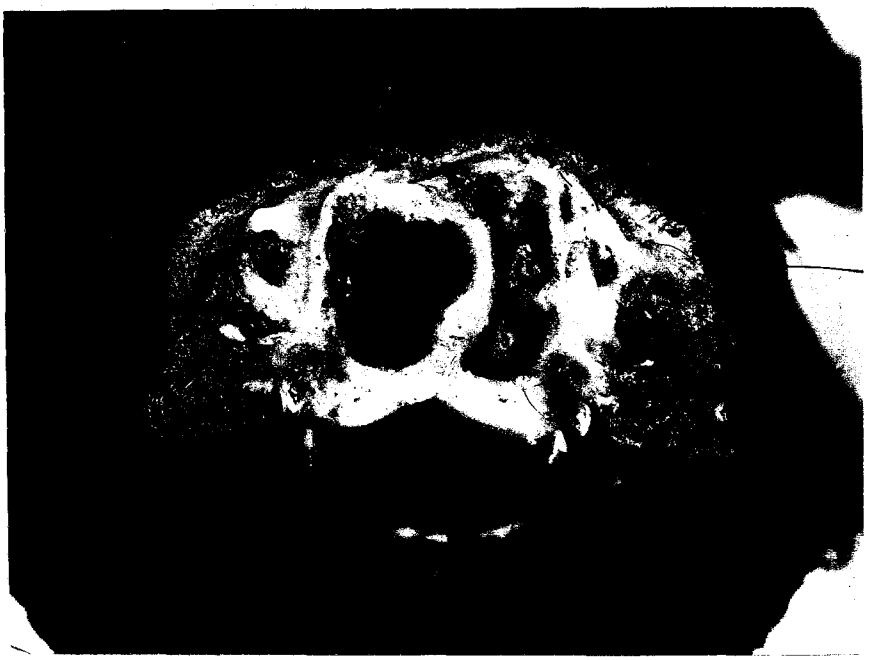

Figure 6. The same pig head as Figure 5 but sectioned to show severe atrophy of one turbinate, mild atrophy of one turbinate and distortion of the nasal septum.

pigs with definite turbinate atrophy had normal lungs. Since pneumonia in both infectious atrophic rhinitis negative and positive swine is quite common, it does not appear possible at this time to evaluate the role infectious atrophic rhinitis plays in the production of pneumonia.

A few pigs with experimentally transmitted infectious atrophic rhinitis developed fibrinous pericarditis, pleuritis, peritonitis and arthritis. These same lesions were occasionally observed in field cases of infectious atrophic rhinitis. However they cannot be considered indicative of this disease since most of the pigs experimentally infected with infectious atrophic rhinitis did not develop these lesions.

Histological examination of nasal turbinates from swine affected with varying degrees of turbinate atrophy revealed that one of the earliest changes that occurs in this disease is a mild infiltration of the submucosa with lymphocytes, lymphoblasts and macrophages. The epithelium was not observed to undergo any significant alteration at this stage of the disease. At about the same time that the initial submucosal infiltration occurs, the osteoblasts, especially those at the extremity of the ventral scroll, show an increase in both numbers and size. This alteration of the osteoblasts sometimes involves a considerable area

but sometimes is confined to very definite regions. The submucosal infiltration is usually apparent along a considerable portion of the turbinate, but is more intense near the end of the ventral scroll of the turbinate.

As the disease increases in severity the osteoblastic dedifferentiation becomes more pronounced and the osteocytes also become dedifferentiated. This results in an increase in the size of the osteocyte lacunae. There is a resorption of the bone salts in the immediate area of the large, dedifferentiating osteoblasts, resulting in small depressions being produced along the margin of the osseous tissue.

As these tissue changes become well established there is an increase in the number of tubuloalveolar glands present in the submucosa. They contain an excessive amount of mucus. There is no apparent alteration in the walls of the blood vessels as the disease develops, except that a slight thickening of the blood vessels occurs due to the shrinkage of the vessels as the reduction in the size of the turbinate occurs.

As the disease advances to a more severe form, the main change that occurs is a more intense dedifferentiation of the osteocytes and osteoblasts into tissue that is histologically indistinguishable from fibrous connective tissue. This change occurs most frequently at the end of the ventral scroll. In some cases it is possible to trace

the normal outline of the osseous tissue by the band of dedifferentiated osteocytes and osteoblasts that have replaced it. Figures 7 and 8 show a normal turbinate while Figures 9 and 10 illustrate the tissue alterations that occur in infectious atrophic rhinitis.

In severe cases there is some hydropic degeneration of the epithelium, but this usually occurs after there is an accumulation of exudate in the nasal cavity. As exudate accumulates in the nasal cavity many bacteria become established. This results in considerable exfoliation of the nasal epithelium and in extensive infiltration of the nasal submucosa with neutrophilic leucocytes. However, the epithelium shows no evidence of being primarily involved in this disease.

The inferior scroll of the ventral turbinate is the area that usually undergoes the initial, and also the most severe, atrophy. Therefore it is not unexpected that the most pronounced histological alterations usually occur in this region. The resorption of the osseous tissue is found to be associated with the dedifferentiation of the osteocytes and osteoblasts.

Figure 7. The extremity of the inferior scroll of the ventral nasal turbinate of a normal swine. Giemsa's stain, X100.

Figure 8. The same normal turbinate specimen as Figure 7. Note the normal differentiation of the osteoblasts. Giemsa's stain, X420.

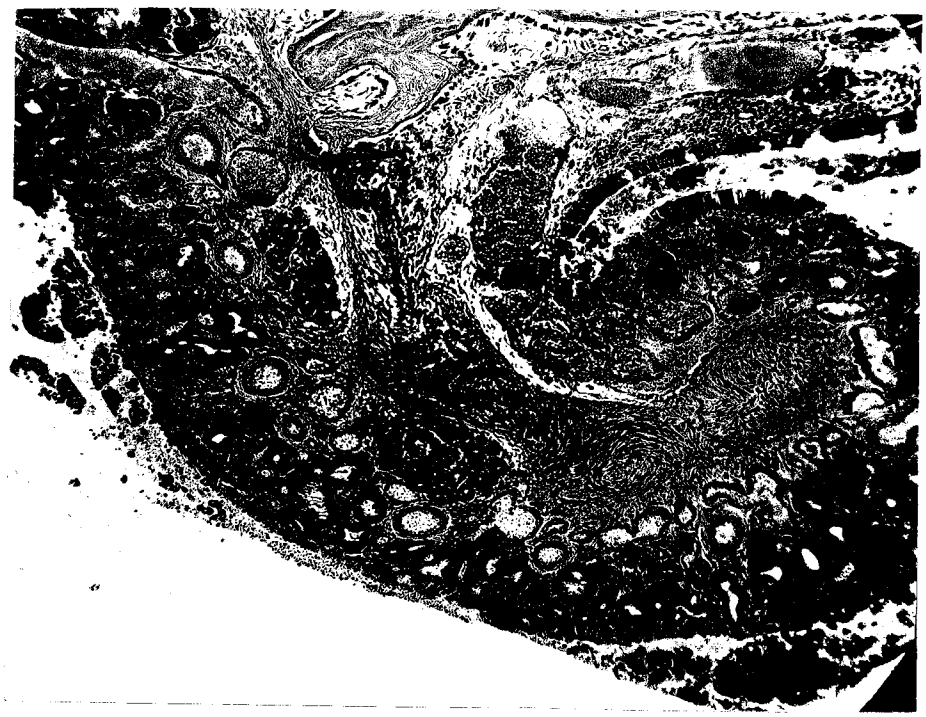

Figure 9. A section of the inferior scroll of the ventral turbinate from a pig with infectious atrophic rhinitis. Note the tissue morphologically indistinguishable from fibrous connective tissue that fills the area normally occupied by the turbinate bone. Giemsa's stain, X100.

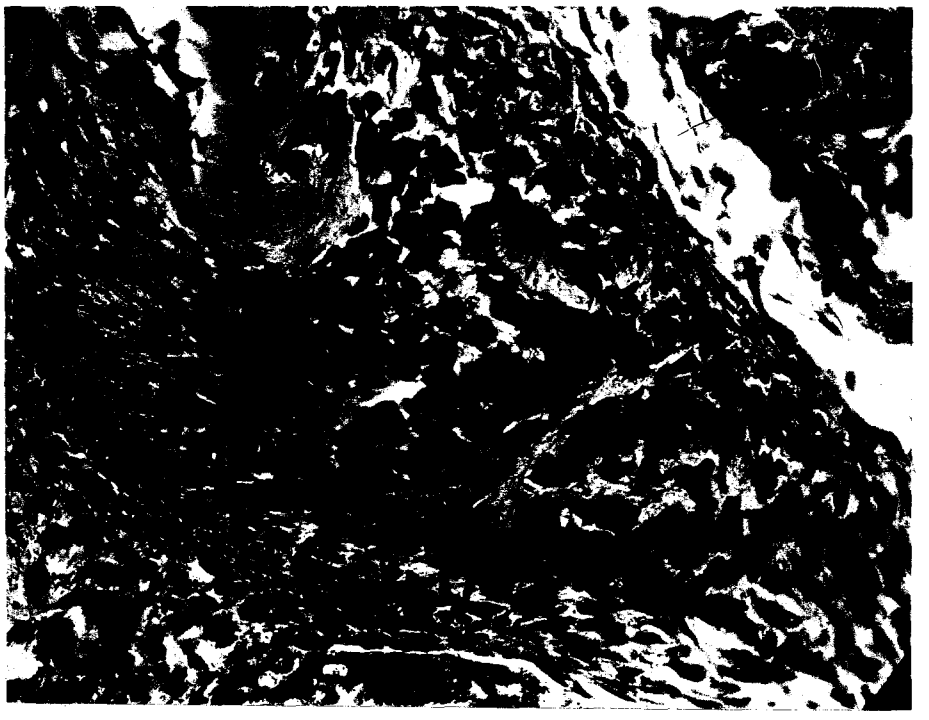

Figure 10. The same turbinate section as Figure 9 but at a higher magnification to show dedifferentiation of the osteoblasts into tissue morphologically indistinguishable from fibrous connective tissue. Giemsa's stain, X480.

Cultivation of the Swine Pleuropneumonia-like Organism in Chicken Embryos

In view of the possibility that an infectious agent may be the cause of the dedifferentiation of the osteoblasts and osteocytes, it was decided to attempt the isolation of some large filterable agent in chicken embryos.

Hofstad (1952a and 1952b) has shown that Selas number 02 porcelain filter candles satisfactorily pass the pleuropneumonia-like organism believed to cause chronic respiratory disease of poultry. Therefore it was decided to prepare filtrates of atrophic swine nasal turbinates using this type of filter. Approximately 0.2 ml. of filtrate was inoculated into the amnioallantoic-sac of each of 12 eight-day embryonated chicken eggs which were subsequently incubated at 37° C. One embryo died on the seventh day and another on the tenth day postinoculation. These two dead chicken embryos showed severe pericarditis and some degree of embryo and yolk sac hyperemia. When the eight remaining chicken embryos were necropsied ten days postinoculation, one had mild pericarditis and anasarca and three embryos had thickened chorioallantoic membranes. It was found that amnioallantoic fluid collected from one of the dead chicken embryos produced

similar lesions when inoculated into another group of 7-day-old chicken embryos. Subsequent study revealed that this agent was a typical pleuropneumonia-like organism.

In this work over 100 isolations of this swine pleuropneumonia-like organism have been made. It was found that this organism produces different lesions in various aged chicken embryos, therefore the age of the chicken embryo must be taken into account when discussing the lesions produced. In addition, only about one-half of the inoculated chicken embryos develop lesions or die, and some develop lesions but do not die. Therefore at least six embryos should be inoculated with each inoculum. When 7- to 8-day-old chicken embryos are inoculated with the swine pleuropneumonia-like organism there is usually no mortality due to this organism during the first four days. Embryo mortality begins on the fifth day and reaches a peak on the sixth or seventh day postinoculation. The death rate diminishes after the seventh day but may continue for as long as 13 days postinoculation. The type of lesion observed in the dead chicken embryos was found to correlate with the age of the embryo at the time of death. In some cases embryos inoculated at 10 days of age and dead four days postinoculation had the same general lesions as embryos inoculated at seven days of age and dead seven days postinoculation.

However embryos inoculated at more than seven to eight days of age were less susceptible to this agent.

Chicken embryos dead on the fourth to the sixth day postinoculation usually had diffuse hyperemia of the skin, the liver and the kidneys. Some of the chicken embryos dead the sixth day postinoculation had mild myocarditis but this lesion was more common in those dead on the seventh day postinoculation. The embryos dead on the seventh day postinoculation usually had a marked decrease in the amount of hyperemia present. The chicken embryos dead from the eighth to the thirteenth day postinoculation usually had severe pericarditis that sometimes was so severe that the exudate could be stripped from the epicardium with forceps. In addition, chicken embryos dead after the eighth day postinoculation frequently had numerous small necrotic foci present in the liver. Figures 11 and 12 illustrate these lesions. All ages of chicken embryos succumbing to this organism had kidney hyperemia. Other lesions occasionally observed in the dead chicken embryos were peritonitis and ulceration of the skin in the occipital region. The chorioallantoic membrane occasionally had areas of cellular infiltration and edema, but this was not a constant lesion. In one chicken embryo a distinct joint abscess occurred.

It was found that aminoallantoic fluid from some inoculated chicken embryos with no gross lesions when necropsied

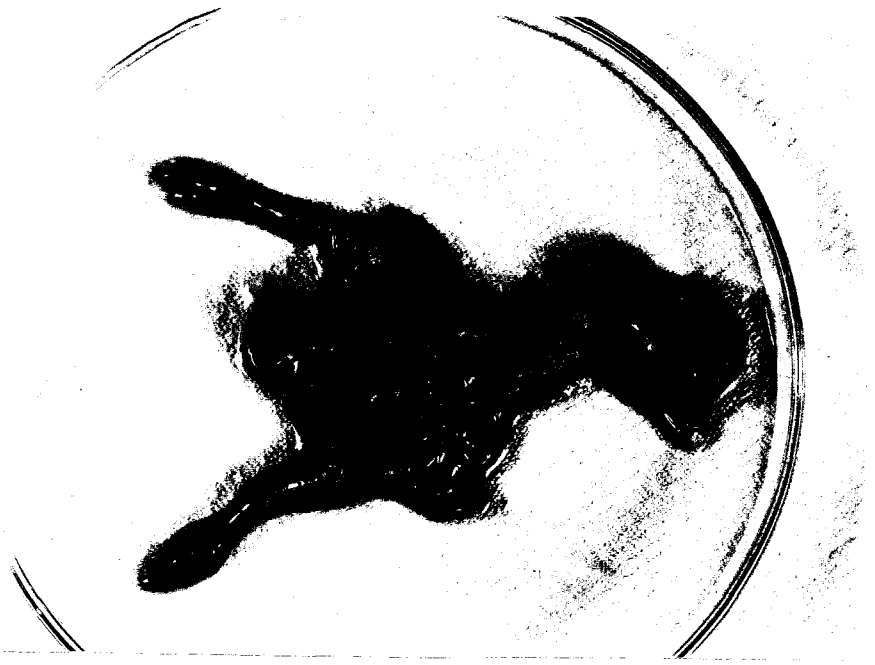

Figure 11. A chicken embryo dead 7 days after inoculation of the swine pleuropneumonia-like organism into the yolk sac. Mild embryo hyperemia, as well as myocarditis and pericarditis, are present.

Figure 12. A chicken embryo dead 10 days after inoculation of the swine pleuropneumonia-like organism into the yolk sac. Severe fibrinous pericarditis and scattered areas of liver necrosis are present.

10 days postinoculation, would produce typical lesions when inoculated into 7-day-old chicken embryos. Therefore in the latter portion of the chicken embryo work, the routine procedure was to make a second chicken embryo passage of amnioallantoic fluid before considering a specimen negative for the swine pleuropneumonia-like organism. Several typical isolates were recovered on the second chicken embryo passage but not the first. In all probability the number of isolates of the swine pleuropneumonia-like organism would have been somewhat greater had this technique been used initially.

To ascertain which chicken embryo material contained the greatest number of the swine pleuropneumonia-like organisms, chorioallantoic membranes, yolk sac membranes, embryos, and amnioallantoic fluid from 11 chicken embryos succumbing to the swine pleuropneumonia-like organism were collected and emulsified. Titration trials using these materials as inoculum were conducted in chicken embryos. It was found that titration trials conducted with this organism in chicken embryos never had a distinct end point. The end point usually extended through three tenfold dilutions. The chorioallantoic membrane had an ID_{50} of $10^{6.5}$, the yolk sac membrane had an ID_{50} of $10^{4.6}$, the embryo proper had an ID_{50} of $10^{4.6}$, and the amnioallantoic fluid had

an ID_{50} of $10^{5.2}$. It will be recognized that it is much simpler to collect chicken embryo amnioallantoic fluid than to collect chicken embryo chorioallantoic membranes. Therefore, amnioallantoic fluid was routinely harvested when material containing the swine pleuropneumonia-like organism was desired.

Cultivation of the Swine Pleuropneumonia-like Organism in Artificial Medium

When smears prepared from chicken embryo pericardial exudate were stained with Giemsa's stain and examined, numerous small coccobacillary objects were consistently observed. Figures 13 and 14 show such smears. The appearance of these objects was extremely suggestive of pleuropneumonia-like organisms. Therefore a prolonged effort was made to grow this organism in artificial medium. After the initial reports of this present work (Switzer 1953a and 1953b) were published, Carter (1953b) verified the existance of a pleuropneumonia-like organism in the nasal cavity of swine and reported that he was able to cultivate it in thioglycollate medium enriched with horse serum. In a few trials using his technique, it was not possible to obtain satisfactory growth. Previous to Carter's (1953b) report

Figure 13. Pericardial exudate from a chicken embryo dead 9 days after inoculation with the swine pleuropneumonia-like organism. The small coccobacillary objects are swine pleuropneumonia-like organisms. Giemsa's stain, X980.

Figure 14. Pericardial exudate from a chicken embryo dead 9 days after inoculation with the swine pleuropneumonia-like organism. The small coccobacillary objects are swine pleuropneumonia-like organisms. Giemsa's stain, X980.

most of the commercial preparations recommended for the cultivation of pleuropneumonia-like organisms had been tried unsuccessfully. Therefore it was decided to try the fresh chicken meat infusion plus chicken serum medium that Hofstad (1954) had developed for the cultivation of the pleuropneumonia-like organism associated with chronic respiratory disease of poultry. It was possible to obtain growth of the swine pleuropneumonia-like organism in this medium. Subsequent work evolved a medium that was considered to give more satisfactory growth of this agent.

Cultures of the swine pleuropneumonia-like organism were obtained in this medium from both filtrates (Selas number 02 filter) of nasal turbinates and crude turbinate material treated with penicillin and thallous acetate. It was found that 48 hours was a satisfactory incubation period. However, if the inoculum was slightly opaque it would make the medium turbid enough that growth of the pleuropneumonia-like organism could not be detected. Therefore it was necessary to wait until the second or third transfer to observe growth of the organism. When stained smears were prepared before the second or third transfer of the cultures inoculated with treated crude inoculum there were frequently enough tissue fragments present that the organisms were difficult to observe. It was desirable

to transfer this organism at 48 to 72 hour intervals. The usual amount of inoculum transferred each time was 1 ml. After seven days incubation without transfer, cultures of this organism were usually nonviable.

In some 24-hour-old cultures growth was present only in the upper portion of the medium. By 48 hours the turbidity was uniform throughout the medium. No pellicle formation was observed in any of the isolates of this organism although there sometimes was a small amount of sediment present. It was advantageous to view the cultures in a strong light looking towards a dark background to see the turbidity. A control tube was always included in each transfer for comparison.

No acid or gas formation could be detected when one per cent lactose, sucrose, dextrose, maltose or mannite plus 0.01 per cent phenol red was added to the fresh ox heart infusion-chicken serum medium. Since pleuropneumonia-like organisms may not produce detectable pH change, it was decided to use the technique of Somerson and Morton (1953) and Lecce and Morton (1954) of adding triphenyltetrazolum chloride to act as an electron acceptor. This compound is reduced to a red formazan compound by some pleuropneumonia-like organisms. Enough of this compound to make a final concentration of 0.005 per cent was added to each of the previous media. The media were inoculated with 1 ml. of a

48-hour culture of the swine pleuropneumonia-like organism and incubated at 37.5° C. for 48 hours. It was observed that more formazan production occurred in the base medium containing no carbohydrate. This suggests that the carbohydrate enrichments actually had a slight inhibitory effect on the growth of this organism.

It was possible to cultivate this organism on solid medium. The colonies were very minute. At approximately 48 hours they imparted a velvet-like appearance to the surface of the medium. These colonies could be readily observed under X20 magnification. The diameter of the colonies was from 0.01 mm. to 0.1 mm. Not all of the colonies developed at a uniform rate. A week after inoculation a few colonies were still developing. This may cause confusion when attempting to determine the presence of more than one pleuropneumonia-like organism in a specimen. In fact it was observed that cultures of this organism could sometimes be initiated in fluid medium from an inoculating needle touched to the surface of a 48-hour culture of this organism, although no colonies visible under 20X magnification were contacted by the inoculating needle. The colony of the swine pleuropneumonia-like organism is small and glistening with regular margins. They have little tendency to coalesce. There is an indefinite central elevation that becomes granular in

appearance as the colony ages and can usually be differentiated from the periphery. The colonies remain distinct and have no tendency to spread or to form films. Figure 15 shows a colony of the swine pleuropneumonia-like organism.

Morphology of the Swine Pleuropneumonia-like Organism

When smears of this organism are stained with Giemsa's stain and examined at X980, it is observed that the predominant form is a minute coccoid rod from 0.3 micron to 0.6 micron in length and 0.3 micron or less in width. Rod forms up to 1.0 micron in length and some small dense spherical forms about 0.2 micron in diameter are observed. In addition, ring forms from 0.3 micron to 0.6 micron in diameter with a definite decrease in the density of the central portion, or an actual vacuolated central portion are observed. Figure 16 shows the appearance of the swine pleuropneumonia-like organism.

When preparation of the swine pleuropneumonia-like organism are examined with the aid of the electron microscope, it is found that the organisms are irregular, flattened spheres many of which have central vacuoles. The organism has little rigidity of the cell wall as

Figure 15. Colonies of a 9-day-old culture of the swine pleuropneumonia-like organism. X120.

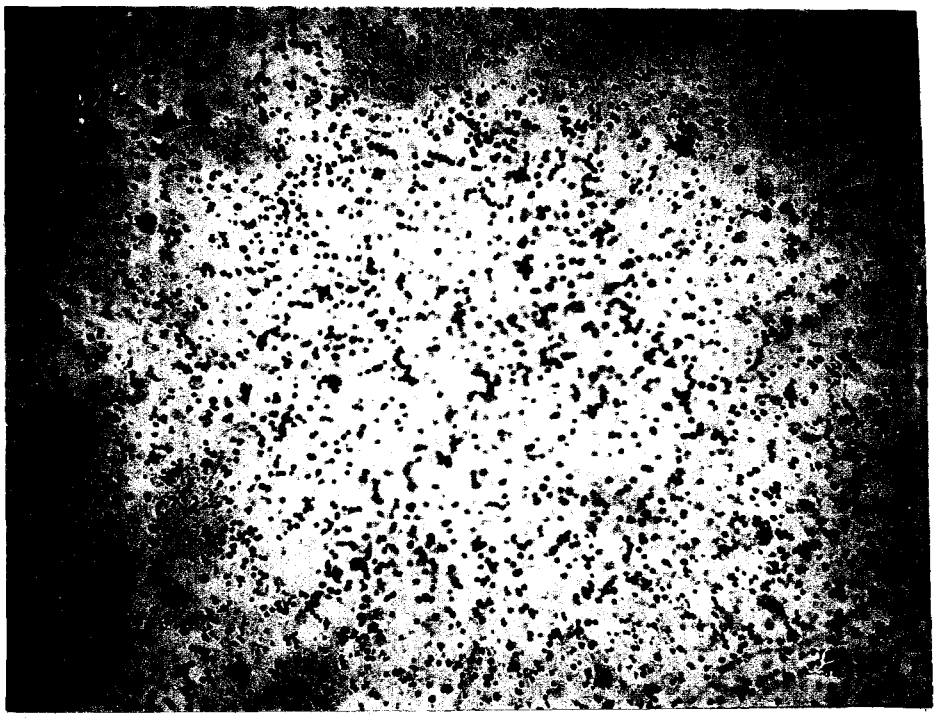

Figure 16. Smear of the swine pleuropneumonia-like organism grown in ox heart infusion-chicken serum medium. Giemsa's stain, X980.

evidenced by an unusual degree of flattening. No filamented forms are observed. Figures 17 and 18 show its appearance.

This organism is not acid-fast, is stained very faintly gram negative with Gram's stain, and is stained a blue color by Macchiavello's stain.

Sensitivity of the Swine Pleuropneumonia-like Organism to Antibiotics and Thallous Acetate

Antibiotic sensitivity tests were conducted as a preliminary appraisal of possible therapeutic agents against the swine pleuropneumonia-like organism, and to determine what inhibiting substances would assist in establishing cultures of this organism from unfiltered crude material. The antibiotic to be tested was added to chicken embryo amnioallantoic fluid containing the organism and allowed to stand for 30 minutes at room temperature prior to its inoculation into 7-day chicken embryos via the yolk sac in 0.2 ml. portions. The addition of 1,000 or 10,000 units of the potassium salt of crystalline penicillin G or 50 units of bacitracin to each ml. of inoculum produced no alteration in the pattern of death of the inoculated chicken embryos. However, streptomycin calcium chloride complex

Figure 17. The swine pleuropneumonia-like organism, shadow cast with gold, X5,600.

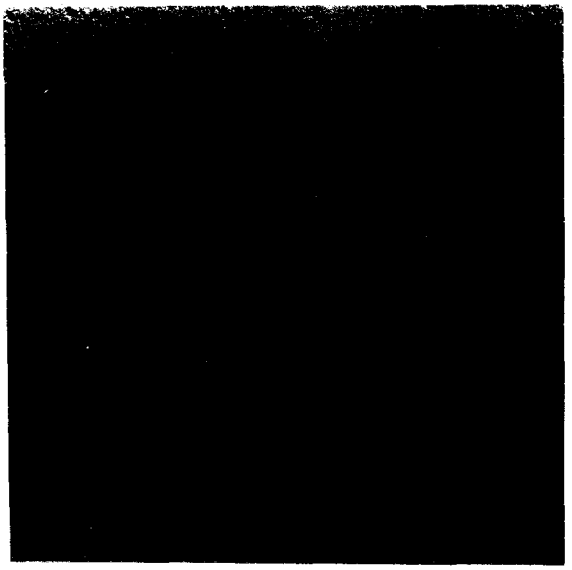

Figure 18. The swine pleuropneumonia-like organism, shadow cast with gold, X30,000.

added at the level of 50 mg. per ml. resulted in survival of 3 of 12 inoculated chicken embryos. Aureomycin hydrochloride with sodium glycinate was added to one inoculum at the rate of 40 mg. per ml. and protected 10 of 12 chicken embryos. The addition of 5 mg. per ml. of crystalline terramycin to one inoculum protected 12 of 12 of the inoculated chicken embryos. The untreated control inoculum proved unusually virulent for chicken embryos, killing 12 of 12.

Morton and Lecce (1953) investigated thallium acetate as a bacterial inhibitor in the cultivation of pleuropneumonia-like organisms and recommended its use. Therefore it was desired to determine its action on the swine pleuropneumonia-like organism. It was found that a 1:2,000 concentration of this material did not influence the growth of cultures of the swine pleuropneumonia-like organism. A 1:4,000 concentration was routinely used to aid in isolating the pleuropneumonia-like organism from crude material. If a large amount of tissue was added to the culture or if the treated material was inoculated into the yolk sac of chicken embryos the inhibitory action of this substance was neutralized and bacterial contamination occurred. An occasional specimen contained organisms that were not inhibited by a 1:4,000 concentration.

Resistance of the Swine Pleuropneumonia-like Organism to Heat and Storage

The swine pleuropneumonia-like organism was found to withstand 56° C. for 30 minutes but not for 60 minutes. It was found to withstand storage at -40° C. for 10 months, which was the longest interval tested. However, cultures of this organism were usually nonviable when stored at 4° C. for three weeks. The addition of 50 per cent glycerine to the cultures stored at 4° C. appeared to have little influence on their survival. Cultures of this organism lyophilized and stored at 4° C. retained their viability for at least a year, which was the longest interval checked. However, there was a considerable drop in the liter of the lyophilized culture material.

Filterability of the Swine Pleuropneumonia-like Organism

The original isolation of this organism was made from a filtrate prepared with a Selas number 02 microporous porcelain filter. This type and porosity of filter was the most satisfactory one used. This organism did not pass

through a Seitz filter equipped with a sterilizing asbestos-wood pulp filter pad. Likewise, a Mandler filter candle of seven pounds bubbling pressure retained this organism. Filtrates of material containing this organism prepared with a Selas number 03 filter had a marked drop in titer when compared to the original material. However, some of the swine pleuropneumonia-like organisms passed through a Selas number 05 filter as evidenced by an occasional chicken embryo inoculated with this filtrate developing typical lesions.

Production of Neutralizing Antibodies against the Swine Pleuropneumonia-like Organism

An attempt was made to elicit neutralizing antibodies against the swine pleuropneumonia-like organism in rabbits, chickens and pigs. Two mature white rabbits were inoculated intravenously with 1 ml. of pooled chicken embryo amnioallantoic fluid containing this organism. The inoculations were made on the 1st, 3rd, 5th, 7th, 10th and 23rd day. Serum was collected from each rabbit on the 48th day after the initial inoculation. This serum was heat inactivated at 56° C. for 30 minutes and mixed with equal amounts of 10^{0}, 10^{1}, 10^{2} and 10^{3} dilutions of chicken

embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism. The mixture was allowed to stand 1 hour at room temperature. Another series of similar tenfold dilutions of the same chicken embryo material containing the swine pleuropneumonia-like organism was mixed with equal parts of sterile tryptose broth to serve as controls. Each dilution of both the rabbit serum-swine pleuropneumonia-like organism, and the tryptose broth-swine pleuropneumonia-like organism was inoculated in 0.2 ml. amounts into the yolk sac of each of nine 7-day-old chicken embryos. No significant difference in the number of deaths or in the lesions present in the chicken embryos inoculated with these two series of material was noted.

A mature rooster was inoculated intravenously with 1 ml. of chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism at the same time intervals as the rabbits. The serum from this bird was tested for neutralizing antibodies using the same technique employed for the rabbit serum. Again, no significant difference was observed between the inoculum diluted with chicken serum and with sterile tryptose broth.

A 4-month-old pig was inoculated with 2.0 ml. of pooled chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism intraperitoneally the

first day, 10.0 ml. in the sixth day, 100.0 ml. in the thirteenth day, and 50.0 ml. in the twenty-sixth day. A serum sample was collected from this pig in the thirty-third day after the initial inoculation and checked for the presence of neutralizing antibodies by the previously described method. Again no significant neutralization was observed.

Inoculation of Animals Other than Swine

The lesions produced by the swine pleuropneumonia-like organism in chicken embryos resembles those produced by the pleuropneumonia-like organism associated with chronic respiratory disease of poultry. Therefore, the determination of the pathogenicity of the swine organism for poultry was desirable.

A total of 20 chickens from 3 to 8 weeks of age and 12 turkey poults from 6 to 8 weeks of age were inoculated intranasally, intratracheally, by way of the infraorbital sinus and by way of the conjunctival sac with chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism. These inoculated birds did not develop respiratory symptoms during a 3-week observation period. No gross lesions were observed in the

respiratory tract when the birds were necropsied at the end of this period.

Chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism was inoculated intraperitoneally, intracrainally, intravenously, and by inhalation of the fluid while under ether anesthesia, into 6- to 8-week-old Carworth Farm Cf_1 and Webster strain white mice. No symptoms were produced and no lesions were observed when the mice were necropsied 11 days postinoculation.

Guinea pigs inoculated intraperitoneally with this organism were normal when necropsied 1 week postinoculation. In addition, the subcutaneous and intravenous inoculation of rabbits with material containing this organism failed to produce any lesions. The rabbits were necropsied from 1 to 7 weeks postinoculation. A 10-week-old calf was inoculated intraperitoneally with material containing this agent. No temperature elevation occurred and no significant lesions were present when the calf was necropsied 2 weeks postinoculation.

A 5-month-old sheep was inoculated intraperitoneally with 5 ml. of a 48-hour ox heart infusion-chicken serum culture of the swine pleuropneumonia-like organism. This sheep developed no temperature elevation and had no lesions present when necropsied 1 week postinoculation.

Intranasal Inoculation of Baby Pigs with the Swine Pleuropneumonia-like Organism

Since the swine pleuropneumonia-like organism was isolated from the nasal mucosa of swine infected with infectious atrophic rhinitis, it was imperative that its effect on the nasal turbinate bones of young, infectious rhinitis-free pigs be determined. In the first four trials pigs were secured from a herd believed to be free of infectious atrophic rhinitis. Each litter was housed in a single room isolation unit with the dam. In the first four trials the pigs nursed the sow until weaning time. In the last trial the pigs were taken from their dam at approximately 18 hours of age and reared by hand in single room isolation units. The pigs were not removed from the isolation units at any time prior to their necropsy. All of the pigs were inoculated intranasally within 5 days after birth. The first four litters of pigs were inoculated with chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism. The fifth litter received an ox heart infusion-chicken serum culture of this organism. One litter was inoculated by exposure to the particle suspension produced in a closed chamber by a Peralta nebulizer operated by 4 to 7 pounds air pressure.

A summary of these inoculations appears in Table 1. Turbinate material was collected from all but one of the inoculated pigs at the time of necropsy and examined for the presence of the swine pleuropneumonia-like organism. Twelve of 17 of these pigs had this organism present at the time of necropsy.

No clinical evidence of any respiratory disease was noted in these inoculated pigs. However, it was noted that several of them developed a mild conjunctivitis as evidenced by excess lachrymation. This condition usually developed from the second to the fourth week postinoculation and was mild enough that it caused the pigs no apparent discomfort.

When this series of intranasally-inoculated pigs was necropsied no distinct atrophy of the turbinate bones was observed. Several of these pigs had an excess of mucous exudate on the nasal turbinate mucosa. Histological examination of these nasal turbinates revealed a mild infiltration of lymphocytes, lymphoblasts and macrophages in the submucosal tissue. The small lymph follicles present in the nasal turbinate submucosa had undergone moderate hyperplasia.

Table 1

Intranasal Inoculation of Baby Pigs with the Swine Pleuropneumonia-like Organism

No. pigs inoculated	Age in days at first inoculation	Inoculum	Method of inoculation	No. of inoculations	Interval from last inoculation to necropsy	Isolate of swine pleuropneumonia-like organism from nasal turbinate at necropsy[a]
3	3	0.5 ml. AAF[b]	Injected intranasally	one	approx. 4 wk.	2 + 1 not ckd.
2	1	1 ml. AAF	Injected intranasally	each day for 6 days	1-6 wk. 1-8 wk.	+ -
4	2	1 ml. AAF	Injected intranasally	each day for 5 days	1-12 wk. 1-14 wk. 1-15 wk. 1-17 wk.	+ + + +
6	2	1 ml. AAF	Nebulized in a closed container for about 15 minutes	each day for 3 days	1-5 wk. 1-10 wk. 4-15 wk.	+ + 4 -
3	2	1 ml. 48 or 72 hr. culture[c]	Injected intranasally	each day for 4 days	5 wk.	3 +

[a]All turbinates normal in appearance.
[b]Amnioallantoic fluid.
[c]Ox heart infusion-chicken serum medium.

Visceral Lesions Produced in Pigs by the Swine Pleuropneumonia-like Organism

A preliminary trial indicated that the swine pleuropneumonia-like organism was capable of producing fibrinous peritonitis, pleuritis, and pericarditis when inoculated intraperitoneally into young pigs. Four 6-week-old pigs were inoculated intraperitoneally with amnioallantoic fluid from chicken embryos dead 8 and 9 days after inoculation with the swine pleuropneumonia-like organism. The amount of inoculum each pig received varied from 0.5 ml. to 4.0 ml. of undiluted amnioallantoic fluid. In all four of the pigs a leucocytosis developed (predominantly a neutrophilia) that reached its peak on the third day after inoculation. The highest total leucocyte count observed was 61,800 per cmm. A fluctuating elevation of the body temperature occurred. The highest temperature recorded for any of the four pigs was 106° F.

Blood was collected aspetically from three of the inoculated pigs on the ninth day postinoculation and injected into the yolk sac of 7-day chicken embryos. The swine pleuropneumonia-like organism was recovered from one of the three samples, indicating that the agent may be transported by the blood stream to various portions of

the body following its intraperitoneal inoculation into pigs. It was observed that one of the pigs developed an arthritis involving one carpal joint. There was a distinct increase in the amount of synovial fluid present in this joint. A portion of this fluid was collected aspetically in the twelfth day postinoculation and injected into the yolk sac of 7-day chicken embryos. The swine pleuropneumonia-like organism was isolated from this joint fluid, indicating that it was the cause of the arthritis.

When the four intraperitoneally inoculated pigs were necropsied 8 to 12 days postinoculation, the outstanding lesion was the severe fibrinous pericarditis. In addition to this lesion, a moderate peritonitis and pleuritis was observed and the anterior mediastinal lymph node was two to three times its normal size. The swine pleuropneumonia-like organism was isolated from these pericardial, pleural and peritoneal lesions in 7-day chicken embryos. These lesions were negative for bacteria when cultured on blood agar and incubated aerobically for 48 hours. All four of the inoculated pigs had pneumonia, but it is difficult to evaluate the significance of this lesion since the source herd had clinical evidence of a mild pneumonia.

Two to 3 ml. of swine pericardial fluid known to contain the swine pleuropneumonia-like organism was inoculated

intraperitoneally into each of four pigs 8 weeks of age. None of these pigs developed a significant temperature elevation or appeared depressed. When these four pigs were necropsied 10 days postinoculation, a few strands of fibrin were observed in the pleural and peritoneal cavity. No other significant lesions were present.

To obtain additional information on the resistance of pigs over 6 weeks of age to this organism, three 7-1/2-week-old pigs were inoculated intraperitoneally with 2 to 3 ml. of chicken embryo amnioallantoic fluid containing the swine pleuropneumonia-like organism. Again it was observed that only a mild temperature elevation occurred. When the animals were necropsied 7 days postinoculation a few strands of fibrin were present in the pleural and peritoneal cavity. A mild pericarditis was present.

In direct contrast to the mild reaction in the 7-1/2 and 8-week-old pigs were the results obtained by the inoculation of pigs under 6 weeks of age. Over 35 pigs less than 6 weeks of age were inoculated intraperitoneally with the swine pleuropneumonia-like organism and all of them developed either singularly or in combination, a severe fibrinous pleuritis, peritonitis or pericarditis. In addition, approximately 20 per cent of them developed an arthritis. It was observed that pigs inoculated within

the first 2 weeks of life developed the most severe lesions. If the pigs were anemic the lesions appeared to be aggravated. Three of the pigs inoculated under 4 weeks of age have succumbed to a combination of pleuritis, peritonitis, pericarditis, anemia, and pneumonia. Some of these inoculated pigs developed a body temperature of 106$^{8°}$ F. In some the fibrinous exudate on the epicardium, pleura, and abdominal viscera has been 0.5 cm. thick. These findings have emphasized the fact that pigs over 6 weeks of age are relatively resistant to the intraperitoneal inoculation of the swine pleuropneumonia-like organism while pigs less than 4 weeks of age develop severe lesions when inoculated intraperitoneally with this organism. Figures 19 and 20 illustrate the lesions produced by this organism.

Recovery of the Swine Pleuropneumonia-like Organism from Field Cases of Swine Pleuritis, Peritonitis, Pericarditis and Arthritis

The lesions present in the pigs inoculated intraperitoneally with the swine pleuropneumonia-like organism resembled lesions occasionally observed in swine submitted to the Iowa Veterinary Medical Diagnostic Laboratory for diagnosis. The etiology of these lesions was usually

Figure 19. Swine visceral lesions resulting from the intraperitoneal inoculation of the swine pleuropneumonia-like organism into a 6-week-old pig. Note the distention of the pericardium with serosanginous fluid, the pleural adhesions, and the scatter areas of fibrinous exudate on the spleen and caecum.

Figure 20. Severe fibrinous pericarditis produced by the intraperitoneal inoculation of the swine pleuropneumonia-like organism into a 6-week-old pig.

unknown. Therefore an attempt was made to isolate the swine pleuropneumonia-like organism from several of these field cases. A specimen from each of 16 different herds with these lesions was examined for the presence of this organism. It was recovered from 8 of 16 (50 per cent) specimens.

Since some of the experimental pigs developed an arthritis following the intraperitoneal inoculation of the swine pleuropneumonia-like organism, synovial fluid from arthritic joints present in each of eight different herds were examined for its presence. This organism was recovered from three of these eight specimens. In addition to recovery of the swine pleuropneumonia-like organism from these lesions, it was recovered from 30 pneumonia lung specimens. It is difficult to evaluate the role of this organism in the production of these pneumonic lesions since it was found that intratracheal inoculation of four 10-day-old pigs under ether anesthesia, with amnioallantoic fluid containing this agent did not produce pneumonia. The animals were necropsied 2 weeks postinoculation. The recovery of the swine pleuropneumonia-like organism from these pneumonic lungs is also minimized by the fact that it was present in the nasal cavities of 18 of the 30 (60 per cent) pigs. Five of the nasal cavities were not examined.

Occurrence of the Swine Pleuropneumonia-like Organism in Normal and in Infectious Atrophic Rhinitis Affected Swine Nasal Cavities

Nasal turbinate specimens from a total of 186 swine have been examined for the swine pleuropneumonia-like organism. A total of 96 of these specimens had gross turbinate atrophy. Of these 96 specimens, 64 (66.7 per cent) were positive for the swine pleuropneumonia-like organism while 32 were negative.

Of 90 grossly normal swine nasal cavities examined, 52 (57.8 per cent) were positive for the swine pleuropneumonia-like organism, while 38 were negative for this organism. A summary of these findings is presented in Table 2.

Characterization of the Swine Pleuropneumonia-like Organism

At this point it appears advantageous to summarize the characteristics of the swine pleuropneumonia-like organism. The natural habitat of this organism appears to be the swine nasal cavity. It is readily filterable through a

Table 2

Occurrence of the Swine Pleuropneumonia-like Organism in the Nasal Cavity of Normal and of Infectious Atrophic Rhinitis Affected Swine

	Turbinates positive for pleuropneumonia-like organism	Turbinates negative for pleuropneumonia-like organism	Total
Swine from field cases			
Atrophic turbinates	18 (69.2 per cent)	8 (30.8 per cent)	26
Normal turbinates	18 (66.7 per cent)	9 (33.3 per cent)	27
Swine from V.M.R.I. herds			
Atrophic turbinates	46 (65.7 per cent)	24 (34.3 per cent)	70
Normal turbinates	22 (47.8 per cent)	24 (52.2 per cent)	46
Swine inoculated intranasally with pleuropneumonia-like organism			
Atrophic turbinates	0	0	0
Normal turbinates	12 (70.6 per cent)	5 (29.4 per cent)	17

Selas number 02 filter and can be grown in serum enriched fluid medium and on suitable solid medium. It produces colonies from 0.01 mm. to 0.1 mm. in diameter with an entire periphery and a smooth glistening appearance. These colonies have an indistinctly elevated central portion that becomes granular as the colony ages. The individual organisms appear as minute coccoid rods 0.3 to 0.6 microns in size and are frequently vacuolated. This organism is stained by Giemsa's stain but not by Gram's stain. Macchiavello's stain imparts a distinct blue color to this organism. It is relatively insensitive to penicillin, bacitracin, or 1:4,000 concentration of thallous acetate. It is slightly susceptible to streptomycin; and it is relatively susceptible to aeuromycin and terryamycin. This organism produces no detectable acid or gas from lactose, sucrose, dextrose, mannite, maltose or 0.5 per cent athyl alcohol. This organism produces an irregular mortality pattern when inoculated into chicken embryos. The outstanding lesion seen in the chicken embryos succumbing to this organism is the severe pericarditis that is usually present in those dead after the seventh day postinoculation. The organism will withstand 56° C. for 30 minutes but not for 1 hour, will remain viable for 2 to 3 weeks at 4° C., and for more than 10 months at -40° C. It can be preserved by lyophilization. The

organism does not produce an appreciable level of neutralizing antibodies in chickens, rabbits or pigs. When the swine pleuropneumonia-like organism is inoculated intraperitoneally into pigs 6 weeks or less of age, it produces a severe fibrinous pericarditis, a moderate fibrinous pleuritis and a mild peritonitis. In addition from 5 to 20 per cent of the inoculated pigs develop an arthritis. Similar lesions produced by this organism occur in field swine.

Relationship of *Pasteurella multocida* and *Spherophorus necrophorus* to Infectious Atrophic Rhinitis

Several reports have been published which indicate *Pasteurella multocida* alone or in association with *Spherophorus necrophorus* produces turbinate atrophy in swine. An attempt was made to evaluate the role of these organisms in the production of turbinate atrophy in the small infected herd maintained for infectious atrophic rhinitis research. The nasal turbinates from three infected litters, totaling 17 pigs, were examined for the presence of *Pasteurella multocida* and *Spherophorus necrophorus*. Atrophic turbinate material from the first 16 pigs produced no lesions when injected subcutaneously

into rabbits but the last specimen produced subcutaneous necrosis typical of Spherophorus necrophorus. A portion of each atrophic turbinate suspension was cultured aerobically on blood agar. No Pasteurella multocida were recovered from any of the 17 atrophic turbinates even though one atrophic turbinate suspension from each of the litters produced turbinate atrophy when inoculated intranasally into a litter of baby pigs.

DISCUSSION

The work of Switzer (1953a) is the first report of the occurrence of a pleuropneumonia-like organism in swine, although Carter (1953) reported the occurrence of a similar if not identical organism in swine soon afterwards. When work on the swine pleuropneumonia-like organism had progressed to the point that it became apparent it would produce arthritis in swine, a similarity was noted to an agent isolated from swine arthritis cases by McNutt et al. (1945). However the characterization of the organism McNutt et al. ioslated was not sufficient to allow satisfactory comparison. No culture of their oganism was available.

Sabin (1941) summarized the criteria for admission of an organism into the pleuropneumonia group as follows:

> The criteria which admit a microorganism into the pleuropneumonia group are: (1) growth in cell-free media with the development of polymorphic structures including "rings", globules, filaments and minute, filterable elementary bodies, usually 125 to 250 mμ in size, which are the minimal reproductive units; and (2) the development on suitable solid media of characteristic minute colonies which may be as small as 10 to 20 μ and as a rule not larger than 600 μ . These characteristics are shared by the saprophytic as well as the parasitic members of the group, but the latter are further

distinguished by their inability to grow in cultures that do not contain a high concentration of serum protein.

These criteria are fulfilled by the organism described in this work. All organisms belonging to the pleuropneumonia group except the organism causing bovine pleuropneumonia are referred to as pleuropneumonia-like organisms. These facts form the basis for considering the organism reported in this work to be a pleuropneumonia-like organism. In addition this organism does not revert to a bacterial form which indicates that the organism is not an L-form of a bacterium.

The swine pleuropneumonia-like organism is relatively fastidious. It grows well in the ox heart infusion-chicken serum medium but will grow equally well when 1 per cent Difco serum fraction is substituted for the chicken serum. The substitution of horse or swine serum in place of the chicken serum did not give satisfactory growth. It was found that pork heart infusion could be substituted for the ox heart infusion in this medium but it imparted an objectionable opalesence to the medium. When the organism was grown in pork heart medium it was slightly more dense and coccoid in appearance.

The initial isolations of the swine pleuropneumonia-like organism were made from the nasal turbinates of swine

affected with infectious atrophic rhinitis. This stimulated speculation that this organism might be of etiological significance in this disease. However the results obtained in this work indicate that this organism alone does not produce turbinate atrophy and that its presence is not correlated with turbinate atrophy. The failure of the swine pleuropneumonia-like organism inoculated intranasally into baby pigs to produce turbinate atrophy was not due to failure of the organism to become established since it was recovered from the nasal cavities of 12 of 17 of the inoculated pigs at the completion of the observation period. In one case this was 4-1/2 months after the last inoculation. Furthermore it has been found (Switzer 1954b) that filtrates (Selas number 02 filter) of atrophic turbinates which contained the swine pleuropneumonia-like organism did not produce turbinate atrophy when inoculated intranasally into baby pigs. This indicates that the failure of baby pigs to develop turbinate atrophy when inoculated intranasally with this organism is not due to loss of virulence of the organism because of cultivation in artificial media.

The swine pleuropneumonia-like organism is commonly present in the nasal cavity of Iowa swine since 116 of 186 (60.3 per cent) swine nasal cavities examined were

positive for the organsim. This organism is probably spread from the nasal cavity of one pig to the nasal cavity of another by inhalation of aerosols containing the organism.

Some of the pigs inoculated intranasally with this organism developed fibrinous pericarditis, pleuritis and peritonitis. This suggests that some pigs develop visceral lesions as the result of the organism gaining entrance to the blood stream from the nasal cavity. The relatively common occurrence of visceral lesions in some field herds suggests that some stress factor has lowered the resistance of the animal and allowed the organism to gain entrance into the blood stream. The history of some of these herds indicates that infectious atrophic rhinitis may be one of the diseases that predisposes to invasion by this organism.

The lesions produced in the young pigs inoculated intraperitoneally with the swine pleuropneumonia-like organism resemble the condition referred to by Hutyra et al. (1946) as Glässer's disease. This is described as a disease of young pigs that appears after the animals have been exposed to cold and to prolonged transport. It is characterized by sero-fibrinous or fibrino-purulent pleuritis, pericarditis, peritonitis and occasionally multiple arthritis. The etiology of this disease has been

investigated by several workers. Hjärre and Wramby (1943) considered that Hemophilus suis was the cause of this condition and Bakas et al. (1952) offered confirmation of this finding. However they had to inoculate a large amount of a culture of this organism to produce the visceral lesions.

No Glässer's disease material from Europe was available for examination in this study so the occurrence of the swine pleuropneumonia-like organism in this disease syndrome could not be determined. However there appears to be no significant difference between the disease syndrome referred to in this country as fibrinous pericarditis, pleuritis and peritonitis and the disease syndrome referred to in Europe as Glässer's disease. Therefore it appears quite possible that the swine pleuropneumonia-like organism is concerned in the etiology of the Glässer disease.

Schofield (1948) and Schofield and Jones (1950) have reported the histological alterations they observed in the nasal turbinates of swine affected with infectious atrophic rhinitis. Many of their observations are in agreement with those made in this study. They noted that there was usually an infiltration of large lymphocytes into the nasal submucosa, an increase in the number of tubulo-alveolar glands and no significant alteration of the lining epithelium of the nasal turbinate. In addition it was

reported by Schofield (1948) and Schofield and Jones (1950) that the wall of the blood vessel had no detectable alteration until late in the disease when it became thickened. It was postulated that this was due to shrinkage of the area of the vessel bed as the turbinate underwent atrophy.

The present investigation indicated that essentially these same changes were present in the atrophic turbinates that were examined. It was observed that lymphocytes, lymphoblasts and macrophages were the predominant cells infiltrating the submucosa. Schofield (1948) and Schofield and Jones (1950) observed that one of the earliest changes in this disease was a proliferation of the osteoblasts. In these areas of proliferating osteoblasts there was usually a rarification of the bone. In advanced cases they observed that the osteoblasts were present in enormous numbers and filled the space left by the disappearing bone. They regarded this as evidence that the osteoblasts were putting forth an heroic effort to rebuild the bone. The observations made during the present study have lead to an entirely divergent evaluation of the relationship of the proliferating osteoblasts to the disappearance of the osseous plates and trabeculae of the nasal turbinate bones.

In the work of Wilton (1937) the active resorption of bone by dedifferentiation of osteoblasts due to some

alteration of the normal state of these cells is summarized as follows:

> The dissolving process occurs first in the neighbourhood of the nuclear area, whereby cytoplasm is demasked and the nuclear area becomes enlarged. In a rapid course of osteolysis, the matrix may be entirely dissolved over connected areas, in which case the bone protoplasm, on being demasked, appears either as connecting multinucleate masses of protoplasm (thrypsis bone giant cells) or as circumscribed anastomosizing uninuclear cells.
>
> When the matrix is dissolved and the bone cells have come to lie outside the calciferous bone, the peripheral parts of the cytoplasm seem to lose their cytoplasmic character, and form a structureless mass, recalling exoplasm in less differentiated bone. In connection with this alteration, the uninuclear cells become more spindle-like and finally assume a morphological type which cannot be distinguished from ordinary connective cells. The nuclei become bladder-like with a granular chromatin network as in less differentiated bone cells. In connection with the above described nuclear alterations, there often takes place a division of the cytoplasm. The uninuclear cells arising by the first-mentioned process may afterwards also divide. As a result of the last-mentioned phenomenon, the cells which previously were uninuclear become multinuclear. There also arises an increase of the nuclei in the bone giant cells. In connection with the division of the nuclei, there can also be constricted off from the giant cells, uninuclear cells which are capable of cell division. As a result of the above-described nuclear process there arises a tendency to proliferation in the dedifferentiated decalcinated bone.

This description appears to adequately explain the histogensis of the bone lesions observed in the nasal turbinates examined in this work. The interpretation of

Schofield (1948) and Schofield and Jones (1950) that the osteoblasts were putting forth an heroic effort to repair the damaged bone but never seemed to succeed is not compatable with the osteoblastic alterations observed. It is apparent that some agent or agents affect the osteoblasts and osteocytes of the boney plates and spicules of the nasal turbinate bones and cause them to dedifferentiate. This results in the resorption of the calciferous deposits contained in the cytoplasm of these cells. As the osteocytes and osteoblasts dedifferentiate they proliferate. It is often possible to trace the normal outline of the re-absorbed nasal turbinate bone by the tract of dedifferentiated osteocytes and osteoblasts that appear morphologically indistinguishable from fibrous connective tissue. It has not been determined that the dedifferentiation of these cells is due to the actual presence in the cell of some infectious agent. However no bacteria were observed in the areas of dedifferentiation in any of the tissue sections examined in this work.

Recently several investigators have reported that cultures of Pasteurella multocida produce turbinate atrophy when inoculated intranasally into young pigs. It is apparent that this organism was not responsible for the turbinate atrophy observed in the small herd of infectious

atrophic rhinitis infected swine maintained for this work since it was recovered from none of the atrophic turbinates. In addition the presence of Spherophorus necrophorus in 1 of 17 atrophic turbinates indicates that it is not the primary cause of turbinate atrophy in this herd.

The possibility exists that more than one agent may be found to cause turbinate atrophy. It is possible that the divergent results obtained by various research workers investigating this disease will correlate better when we have more information available.

One main factor which has slowed the work on infectious atrophic rhinitis is the lack of a laboratory animal susceptible to the disease. An effort was made to determine if 12 one-day-old mice might evidence turbinate changes following their intranasal inoculation with crude atrophic turbinate material. At the end of three weeks their nasal cavities appeared normal. In addition 3 seven-week-old kittens were inoculated intranasally with crude atrophic turbinate material on each of five consecutive days. At the end of four weeks there was no detectable difference between the nasal cavities of the three inoculated kittens and two uninoculated littermates. Up to the present time no suitable laboratory animal has been found to replace the baby pig in the study of this disease.

The work presented here and the confirmation found in the work of Carter (1954) brings to five the number of diseases of domestic animals and poultry known to be due to pleuropneumonia and pleuropneumonia-like organisms. These five diseases are contagious pleuropneumonia of cattle; contagious agalactia of sheep and goats; contagious pleuropneumonia of goats; chronic respiratory disease of poultry; and swine pericarditis, pleuritis, peritonitis and arthritis.

CONCLUSIONS

1. The preliminary report of this work published by Switzer (1953) is the first report of the occurrence of a pleuropneumonia-like organism in swine.

2. The results obtained in this work indicate that the intranasal inoculation of the swine pleuropneumonia-like organism into baby pigs does not produce turbinate atrophy and that the occurrence of this organism in the nasal cavity of swine is not correlated with turbinate atrophy.

3. The swine pleuropneumonia-like organism produces, either singularly or in combination, pericarditis, pleuritis, peritonitis and arthritis when inoculated intraperitoneally into young pigs.

4. Similar lesions due to this organism occur in field swine.

5. The swine pleuropneumonia-like organism can be grown in chicken embryos or in ox heart infusion-chicken serum medium.

6. Rabbits, guinea pigs, mice, chickens, turkeys, a calf, and a sheep developed no lesions when inoculated with the swine pleuropneumonia-like organism.

7. Pasteurella multocida and Spherophorus necrophorus were not responsible for the turbinate atrophy in the infected herd studied.

8. The basic lesion of infectious atrophic rhinitis is the dedifferentiation of the osteoblasts and osteocytes of the turbinate bones.

9. Day-old mice and 7-week-old kittens are not susceptible to infectious atrophic rhinitis.

10. The etiology of infectious atrophic rhinitis of swine is still unknown.

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