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CONTENTS

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J. A. HOWARTH

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SPIROCHETES AS THE ETIOLOGICAL FACTOR IN CERTAIN SPECIFIC NECROSES AND HYPERPLASTIC FORMATIONS IN SWINE

J. A. HOWARTH1

The high percentage of young swine on the Pacific Coast of the United States affected with rhinohyperplasia² and scirrhous cord (neoplastic formations in the scortum following castration) has been the incentive for some investigations, which are herewith reported.

Published observations concerning a form of rhinitis in swine called 'snuffling sickness' appeared in the German veterinary literature as early as 1860. Haubold,⁽¹⁾ Harms,⁽²⁾ Wulff,⁽³⁾ and Ostertag,⁽⁴⁾ considered the cause to be a rachitic swelling of the ethmoid and sphenoid bones. According to Schell,⁽⁵⁾ sarcomatous growths produced the symptoms, while Haubner⁽⁶⁾ considered it a form of tuberculosis, and Damman⁽⁷⁾ believed some cases to be caused by actinomyces, and others by abnormal development of the nasal bones. Schneider⁽⁸⁾ reported a form of 'snuffles' caused by the rudimentary development and curvature of the turbinated and ethmoid bones leading, in some cases, to a bloody purulent nasal discharge. Iminger,⁽⁹⁾ after observing a large number of cases, decided that the disease was infectious. He reported cases in which the cerebral tissues were involved.

Friedberger and Frohner⁽¹⁰⁾ pointed out that several of the German authors had described a disease of pigs which is evidently not uniform in character, since sometimes the primary ailment seemed to be rickets, and at others a chronic hemorrhagic, suppurative nasal catarrh pos-

¹Associate in Veterinary Science.

² This disease has been called bull nose, snuffles or necrotic rhinitis by veterinarians and swine owners in the United States.

sibly of an infectious nature. Friedberger and Frohner also classed rhinitis in swine under two forms (a) catarrhal and (b) rickety. Koske⁽¹¹⁾ attributed an infectious form which he studied, to *Bacillus pyocyaneus*; and, since that time, most writers on the subject have given that organism as the cause of infectious rhinitis, and rachitis as the cause of noninfectious rhinitis. These writers include Hutyra and Marek,⁽¹²⁾ Craig,⁽¹³⁾ and Lynch.⁽¹⁴⁾ Kinsley,⁽¹⁵⁾ however, reviewing the German publications under the title, "Infectious Nasal Catarrh," describes acute and chronic forms of rhinitis, both of which he attributes to the inhalation of dust or other irritating substances, to sudden changes in temperature, or to association with other diseased conditions, particularly swine plague and hog cholera.

In 1917, a leaflet, "Necrobacillosis in Pigs,"⁽¹⁶⁾ was issued by the United States Bureau of Animal Industry in which *necrotic rhinitis* was listed among the pathological conditions considered to be necrobacillosis, and the statement was that "the origin of all forms of necrobacillosis is the *Bacillus necrophorus*." In the following year, Graham⁽¹⁷⁾ described necrotic rhinitis, 'sniffles' or bull nose as being caused by *Bacillus necrophorus*.

Some bacteriological examinations of neoplastic tissue from the sinuses of the head, from the snout and from the scrotal region of swine were made by the writer in 1926–1927 at the School of Veterinary Medicine, State College, Pullman, Washington. In every case, a microscopic examination showed the presence of spirochetes. At the University of California, Branch of the College of Agriculture at Davis, abundant material became available, and here, also, examination of the neoplastic tissue from a large number of pigs always revealed the presence of spirochetes. However, the writer found indications that the spirochetes might be the specific cause of both rhinohyperplasia and scirrhous cord in swine. These organisms, in addition to being always present, were observed close to the line of necrosis and also deeper in the neoplastic tissue than other organisms.

In 1894, Theobald Smith⁽¹⁸⁾ submitted a report entitled "Coarser and Finer Spirilla in the Intestines of Hogs." Twelve years later, Dodd⁽¹⁹⁾ described a disease of the pig due to a spirochete, which he considered the cause of superficial bodily ulcers that averaged about three-fourths of an inch in diameter. These spirochetes varied in length from 9 to 26 microns, the average being from 14 to 16 microns, and with from 2 to 6 spirals. Cleland⁽²⁰⁾ of West Australia published in 1908 an article on spirochetes in castration tumors of pigs. This spirochete was 10 to 24 microns long, with 2 to 6 spirals. King and Baeslack⁽²¹⁾ in 1912, published a report stating that they found spirochetes in the blood of hogs suffering from hog cholera. King, Baeslack, and Hoffman⁽²²⁾ in 1913, observed spirochetes in the blood of hogs infected with cholera. This organism averaged from 5 to 7 microns in length and 1 micron in width. Later in the same year, King and Hoffman⁽²³⁾ published an article entitled "Spirochaeta Suis as a Pathogenic Organism in Hog Cholera." Nomi and Matsuo⁽²⁴⁾ published in 1922 their studies on spirochetes in swine, and in a synopsis of the work in English at the end of the article divided the organisms into two general groups as follows: "One is of a very fine spiral form about 10 to 15 microns in length, and some of the organisms belonging to this group are 27 microns long, but they seem to have developed into a stage of division. The spirals are irregular and vary in number from 2 to 8 or even more. The spirals are 0.5-5 microns long and 1-2microns deep. The dimensions of the body are too small to allow of measurement, but appear to be about $\frac{1}{4}$ micron. The other is 10-15 microns long and twice as wide as the former and well observed by the use of Giemsa's stain. The spirals are relatively regular and about 10 in number. The length and depth of the spirals are 1 micron." Schmid⁽²⁵⁾ in 1925, described a disease occurring in southwest Africa in which pigs were affected with lesions on the skin and tumor-like swellings on different parts of the body. Microscopic examinations of material from these lesions showed immense numbers of spirochetes. Descazeaux⁽²⁶⁾ in 1926, reported finding spirochetes in affections of pigs in Chile. He was unable to reproduce the affection by intracutaneous or subcutaneous inoculations of healthy pigs.

GROSS APPEARANCE OF THE NEOPLASTIC FORMATIONS AND RHINOHYPERPLASIA

In the cases studied by the writer, the tumor-like formations located in the region of the castration wounds varied from 2 cm to 30 cm in diameter. The weights of these formations after surgical removal varied from 2 ounces to $221/_2$ pounds. In the early stages, the mass was very firm; but later, fluctuating areas were found, upon palpation. Abscess formation took place, later breaking down and forming fistulous tracts to the outside. Foul-smelling fluid was constantly emitted, which dried over the exterior, forming a crust of varying thickness. In these cases, the fluid was enclosed and caused even more intense destruction of tissue.

In the cases of rhinohyperplasia, the face was more or less distorted. There was a severe inflammation of the membranes lining the nose,

and in some cases gangrenous and necrotic areas were found. There was a great thickening of the snout; and, in the more chronic and longstanding cases, necrosis of the bones was not infrequent. Some animals showed bulging of the bones of the face, due to pressure of the accumulating inflammatory material in the sinuses; and this pressure on adjacent structures caused various anatomical alterations. The most common complication was the obstruction of the nostrils which prevented sufficient passage of air.



Fig. 1. Tumor-like formations located in the region of the castration wounds, the weight of which after surgical removal was 22½ pounds.



Fig. 2. Scirrhous cords after surgical removal; opened to show internal aspects of the growths.

The writer, after a careful study of a great number of cases of uncomplicated rhinohyperplasia, has been unable to reveal any gross skeletal changes, such as beading and bending of the ribs, deformity of the sternum, enlargement of the costochondral junctions or epiphyseal ends of the long bones so characteristic of mammalian rickets. Craniotabes were never demonstrable in the flat bones of the skull. Histological evidence of rickets has never been present in any of the cases examined with this condition. At this Station a study of the effect of light rays and restricted nutritional regimes on skeletal deformities of hogs has been carried on by E. H. Hughes of the Animal Husbandry Division. In this work 75 head of hogs have been maintained for March, 1930]

several months in extremely advanced stages of rachitis without any evidences of rhinohyperplasia having been developed. This would seem to have effectively eliminated the supposition that this condition is caused by or constitutes a complication of rachitis in swine, despite the apparent confusion of the two conditions by the German workers cited in the review of literature at the beginning of this paper.

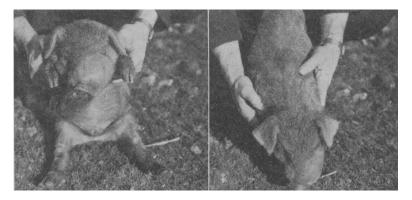


Fig. 3. Rhinohyperplasia showing great thickening of the snout giving the face a distorted appearance.

BACTERIOLOGY

Three distinct organisms were ever present in smears from rhinohyperplasia and scirrhous cord conditions. There were Bacillus subtilis, a common saprophyte in chronic suppurative lesions; Staphy*lococcus puogenes albus*, a secondary invader and a frequent cause of abscesses, boils, and surgical suppurations; and lastly, a spirochete. An unidentified Gram-negative bacillus with terminal spores was present in cultures from only two scirrhous cord cases. It grew seemingly in symbiosis with Bacillus subtilis, and could not be obtained in pure culture. Bacillus necrophorus was isolated from three cases of rhinohyperplasia in all of which the enlargements on the snout had ruptured before the animals were brought to the laboratory. One other animal, pig No. 127, with a scirrhous cord, had an open lesion on the right front leg just above the knee joint. Bacillus necrophorus was isolated from this lesion, but the writer was unable to isolate the organism from the scirrhous cord. This animal afterwards developed an infection of the right front foot, which later ruptured; the material from this lesion was found to contain Bacillus necrophorus. Spirochetes were found present in the scirrhous cord, but not in either lesion on the leg.

Subcutaneous inoculations were made into rabbits with material obtained from the lesions in six scirrhous cord and rhinohyperplasia cases respectively. In cases where *Bacillus necrophorus* was found, the rabbit usually succumbed in from five to seven days. A second and a third rabbit were inoculated in a similar way, with the exception that the material used was from necrotic areas found in the liver of the first rabbit injected. Following this procedure, the writer was able to obtain the necrophorus organism in practically a pure culture.

Bacillus subtilis and the Staphylococcus pyogenes albus were readily isolated in pure culture. The same cannot be said of the spirochete. All attempts to cultivate it anaerobically, on whole blood, blood serum, ascitic agar, and in Hiss serum water, failed. The spirochetes were most successfully demonstrated in direct smears by Giemsa's stain. Almost equally good results were obtained by gentle steaming with carbol-fuchsin for three minutes. The spirchetes exhibited a considerable degree of flexibility, since they straightened out easily in direct smears. They displayed great regularity in their convolutions when stained in the tissue by Levaditi's or by Warthin's methods. In no case did the spirochetes show a tendency for circle formation, V, or Y shapes; neither did they present the right angle bending so characteristic of *Treponema pallida*. The average measurements of the spirochetes were as follows:

Direct smears	$In \ tissue$
Length 9 to 10 microns (variations, 6 to 12 microns)	7.37 microns (variations, 4.38 to 10.22 microns)
Number of turns 4 (variations, 3 to 5)	5.88 (variations, 4 to 7)
Spiral depth 0.2 to 0.6 micron	0.86 micron (variations, 0.73 to 1.46
Amplitude 0.6 to 0.8 micron	microns)
Width 0.1 micron	1.15 microns
	0.24 micron

The spirochete ends were blunt rather than tapering. Motility in contents fresh from the rhinohyperplasia and scirrhous cord, as shown by dark field illumination, was of a rotatory nature.

LOCATION OF THE SPIROCHETES

The spirochetes are found in the tissue just where the line of necrosis and hyperplastic tissue meet. They lie in the minute lymph spaces between the cells and the various fibrils. It has been impossible to demonstrate spirochetes in the blood smears made from the general circulation and obtained under conditions preventing any contamination with spirochetes on the skin or mucous membrane. Smears were taken from all the animals affected; and, after an extensive search, it was proved conclusively that their presence in the blood stream would be accidental. Microscopical preparations and cultures were made from the liver, spleen, lymphatic glands, kidneys, bile, and urine, but all attempts to locate spirochetes were unsuccessful.

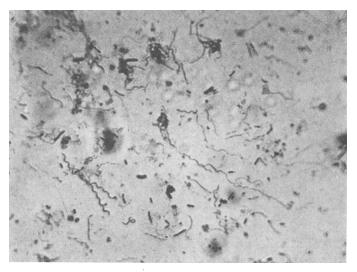


Fig. 4. Spirochetes in smear from scirrhous cord of pig No. 1. Giemsa's stain. Oil immersion, 2 mm. Ocular No. 15.

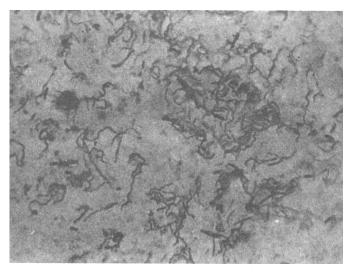


Fig. 5. Spirochetes in smear from rhinohyperplasia of pig No. 149. Giemsa's stain. Oil immersion 2 mm. Ocular No. 15.

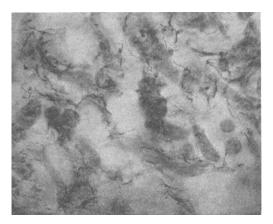


Fig. 6. Spirochetes in tissue lying in the minute lymph spaces between the cells and the various fibrils. Stained by Levaditi's method. Oil immersion 2 mm. Ocular No. 10.

HISTOPATHOLOGY

The neoplasms of inflammatory origin, formed at the end of the cord and tunics, are characterized by a slow proliferative process, due in part to an infiltration and proliferation of the fibroblasts, often combined with lymphocytes, polymorphonuclear leukocytes, and endothelial cells. The peripheral portion of the tumorous mass is made up of a dense, fibrous connective tissue composed of spindle or starshaped fibroblasts, with a considerable variation in the size and shape of the nuclei of the cells, and occasionally with mitotic figures present. The stroma contains collagen and elastic fibers and is edematous in places. There are a large number of arborized blood vessels throughout the mass. Close to the line of demarcation between the hyperplastic tissue and the moist gangrenous area, there is an alteration in the blood vessel walls, and some may be seen plugged with thrombi and leukocytes. Some of the small veins lying in the area close to the line of necrosis show thrombosis; others exhibit areas of localized leukocytic infiltration involving the area between the intima and media, so that the intima is raised and thrown into convolutions until in places its continuity is even broken.

At the line of demarcation between the hyperplastic tissue and the gangrenous area, there is a zone composed of a great number of lymphocytes, polymorphonuclear leukocytes, some fibroblasts, and great numbers of spirochetes. As this zone reaches the gangrenous area, the cells become degenerated, swollen, and granular; the nuclei exhibit karyorrhexis, karyolysis, and pycnosis; and many cells have completely disintegrated. Droplets of fat, bacteria, and cell detritus make up the remaining gangrenous area.

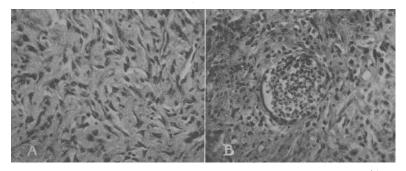


Fig. 7. A, high power view of neoplastic tissue made up of dense fibrous connective tissue, having spindle and star-shaped fibroblasts, collagen and elastic fibers. B, close to the line of demarcation between the hyperplastic tissue and the moist gangrenous area, the blood vessels may be seen plugged with thrombi and leukocytes. High power view.

In rhinohyperplasia, the chronic inflammation of the nares lacks the histological features of a new growth, and in fact many cases showed nothing more than localized edematous areas on the mucous membranes. Over the snout there is a progressive hyperplasia of fibrous connective tissue. In long-standing cases, there is a pronounced

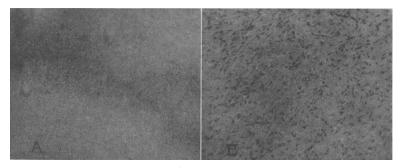


Fig. 8. A, low power view, showing line of demarcation between the necrotic area and the hyperplastic tissue. B, high power view of hyperplastic connective tissue.

inflammation of the periosteal coverings of the bones, later resulting in necrosis and sequestra. Where the sinuses are involved, there is an inflammation and thickening of the mucosa, with an accumulation of necrotic, foul-smelling material, which causes a bulging of the already affected bones.

Throughout the histological examination of all the tumorous masses derived either from scirrhous cord or from rhinohyperplasia, the structure has remained remarkably uniform. There is always that sharp line of demarcation between the gangrenous or necrotic area and the surrounding hyperplastic connective tissue, where the spirochetes are found lying in the minute lymph spaces between the cells and the various fibrils.

ANIMAL INOCULATIONS

Pigs 45 and 46, Duroc Jersey boars, weighing 40 pounds each, in good thrifty condition, were inoculated on August 30, 1928, with 0.1 cc of material containing spirochetes taken from a necrotic area of scirrhous cord from pig No. 1. This infective material was placed into the castration wounds after removal of the testicles. These animals developed scirrhous cords which measured approximately 22–23 cm in diameter on December 4, 1928. Microscopic examination of material discharged from this growth showed the presence of spirochetes.

Pig No. 47, a Poland China boar, weighing 45 pounds, in good thirfty condition, was inoculated on September 4, 1928, with 0.1 cc of material containing spirochetes taken from scirrhous cord of pig No. 3. The infective material was placed into a pocket-like incision on the snout, and 1 cc of the same material was put into the castration wound after removal of the testicles. This animal developed a scirrhous cord and typical rhinohyperplasia. Spirochetes were found present in the necrotic material from both places.

Pigs 11 and 12, both Poland boars, weighing 40 pounds each, were inoculated on September 4, 1928, with 1 cc of material containing spirochetes from scirrhous cord of pig No. 3. Material was placed into an abrasion in the nasal passages. The animals developed enlargements approximately 4 cm in diameter that later ruptured and emitted a foul-smelling material which, on examination, showed the presence of spirochetes. On September 20, 1928, both of these animals showed a characteristic rhinohyperplastic condition. The enlargement was incised, the foul-smelling necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavity. Four days later, a considerable decrease in the size of the swelling was noticed. On December 4, these animals showed very little deformity of the face and had apparently recovered from the infection, the hogs weighing approximately 150 pounds each. Pigs Nos. 17 and 18, Duroc Jersey boars, weighing about 35 pounds each, and in good healthy condition, were inoculated with 0.1 cc of material containing spirochetes taken from necrotic material of pig No. 45 on November 23, 1928. This material was placed into wounds made on the snout. The animals developed characteristic lesions of rhinohyperplasia.

Pig No. 19, a Poland China sow, weighing approximately 25 pounds, in good healthy condition, was inoculated with 0.1 cc of necrotic material containing spirochetes taken from scirrhous cord of pig No. 46. The material was placed in a pocket-like incision in the subcutaneous tissues of the shoulder. Three days later this animal showed characteristic gangrenous lesions, such as is found in scirrhous cord and rhinohyperplasia. There was a discharge of foul-smelling material from the wound, which, upon examination, showed the presence of spirochetes. This gangrenous area continued to enlarge and on December 4 was $4\frac{1}{2}$ cm in diameter.

Pigs 63, 64, and 65, Duroc Jerseys, weighing approximately 30 pounds each and in good health, were inoculated on December 25, 1928, with 0.2 cc of material containing spirochetes obtained from a scirrhous cord and rhinohyperplasia formation. The inoculations were made subcutaneously over the shoulder. On December 30, 1928, swellings about 4 cm in diameter had developed. These swellings finally ruptured, discharging a foul-smelling material which contained great numbers of spirochetes. At the point of inoculation, the lesions increased in size; and on January 10, 1929, they were 9 cm in diameter; the openings to the exterior were 5 cm in diameter. The crater-like lesions were gangrenous, necrotic, and macroscopically comparable to the internal aspect of rhinohyperplasia or scirrhous cord.

Pigs 61 and 62, Duroc Jerseys, weighing approximately 38 pounds each, in good healthy condition, were inoculated on December 25, 1928, with a small piece of tissue removed from a scirrhous cord. This inoculation was made into a pocket-like incision over the shoulder and into the snout. This piece of tissue was taken from the hyperplastic area of scirrhous cord close to the line of necrosis. A portion of this tissue was imbedded, sectioned, and stained; on microscopical examination it was found to contain no organism other than spirochetes. These animals developed characteristic gangrenous lesions, such as are found in scirrhous cord and rhinohyperplasia. Necrotic material taken from these lesions showed, on microscopical examination, great numbers of spirochetes.

Pigs 63 and 64, Poland China, weighing approximately 25 pounds each in good healthy condition, were inoculated on May 7, 1929, with a

pure culture of *Bacillus necrophorus* into two pocket-like incisions over the shoulders. They developed small abscesses 1 to 2 cm in diameter at the point of inoculation, which later ruptured exuding a small amount of thick tenacious cheesy pus. The lesions were in no way similar to those produced by the inoculation of tissue containing spirochetes. On May 27 a necropsy showed no internal lesions, and spontaneous healing had taken place at the point of inoculation, leaving a small amount of scar tissue.

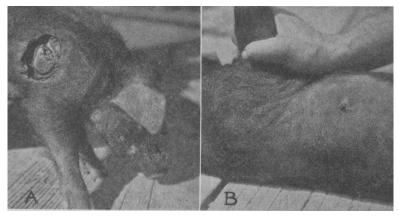


Fig. 9. A, pig No. 61, inoculated subcutaneously over the shoulder and snout with small piece of tissue from scirrhous cord containing spirochetes. B, pig No. 63 inoculated with a pure culture of *Bacillus necrophorus*; spontaneous healing took place at the point of inoculation, leaving a small amount of scar tissue.

A pure culture of Bacillus necrophorus not being obtainable from the "American Type Culture Collection," it was necessary to isolate a pure culture here at this Station. This was done by the following procedure. Necrotic material was taken from the mouth of a calf affected with calf diphtheria, which on microscopic examination contained great numbers of necrophorus organisms. This infectious material was placed into pocket-like incisions on the side of two rabbits, which succumbed in 4 and 6 days, respectively. These animals showed abscesses in the liver, and material taken from these lesions was inoculated into a second series of rabbits, which on autopsy showed abscesses in the liver and kidneys. This procedure was carried on through a third and fourth series of rabbits. Infective material derived from the fourth series of animals was inoculated into gelatin agar media and placed in hydrogen jars at a temperature of 37.5° C. Great numbers of small gas bubbles appeared, followed by small yellowish-white colonies which developed in seventy-two hours.

INOCULATION WITH PURE CULTURES OF ORGANISMS OTHER THAN SPIROCHETES, CULTIVATED FROM SCIRRHOUS CORD AND RHINOHYPERPLASIA

Pig No. 20, a Duroc Jersey boar, weighing 35 pounds, in good healthy condition on November 23, 1928, was thoroughly washed to prevent any contamination of the skin with any spirochetal infection and was placed in a lot that had never contained hogs affected with scirrhous cord or rhinohyperplasia. The animal was castrated and 1 cc of a pure culture of *Staphylococcus pyogenes albus* was inoculated into the wound after the removal of the testicles.

On November 25, a slight swelling was noticed at the seat of castration, which afterwards developed a small abscess; this ruptured, and there was a continual discharge from the wound for several days. The discharge was examined microscopically and found to be free from any spirochetes. This castration wound healed up completely on December 4, with a very slight thickening of the cord and skin at the seat of castration.

Pig No. 21, Duroc Jersey boar, weighing 32 pounds, in good healthy condition, was prepared in the same manner and placed in the same enclosure with pig No. 20. The animal was castrated; 1 cc of a pure culture of *Bacillus subtilis* was inoculated into the wound after removal of the testicles. On November 25, there was a discharge from the wound and some thickening at the margin of the incision. This animal failed to develop a scirrhous cord, and on December 4 appeared perfectly normal.

Pig No. 22, a Duroc Jersey boar, weighing 37 pounds, in good healthy condition, was prepared in like manner, castrated, and inoculated with 1 cc of mixed culture of a Gram-negative bacillus and *Bacillus subtilis* into the wound after the testicles had been removed. On November 25, there was some thickening of the wound and a discharge from the incisions. This animal also failed to develop a scirrhous cord and appeared perfectly normal on December 4.

Pig No. 23, a Poland China boar, weighing 37 pounds, in good healthy condition was prepared in the same manner, castrated, and inoculated with 2 cc of a pooled culture of these organisms, including the *Staphylococcus pyogenes albus*, *Bacillus subtilis*, and a Gramnegative bacillus, into the castration wound after removal of the testicles. On November 25, this animal had considerable discharge

from the castration wounds. There was some thickening and induration of tissue in the locality of the operation. On December 4, this animal had failed to develop a scirrhous cord, and appeared normal with the exception of a small amount of thickening at the seat of castration. Pig 20, 21 and 23, December 26, were sold as healthy animals and free of scirrhous cord.

ATTEMPTS TO TRANSMIT THE SPIROCHETES TO ANIMALS OTHER THAN THE PIG

On August 30, 1928, four guinea pigs (Nos. 1, 2, 3, and 4) weighing approximately 350 grams, in good healthy condition, were castrated; 0.1 cc of necrotic material, containing spirochetes taken from pig No. 1, was inoculated into the wound after removal of the testicles. These animals developed, at the seat of inoculation, abscesses which afterwards were incised; material taken from them was examined microscopically. It was found to contain *Staphylococcus pyogenes albus*, *Bacillus subtilis* and other contaminating organisms, but was negative for spirochetes. These animals failed to produce any gangrenous lesions like those found in the hog, and after repeated inoculations of other guinea pigs, it was impossible to produce a spirochetal infection.

Two buck rabbits (Nos. 99 and 17) in good healthy condition on September 4, 1928, were castrated and 0.1 cc of necrotic material from scirrhous cord of pig No. 3, containing spirochetes, was inoculated into the wound after removal of the testicles. Abscess formation developed. After the lancing of these enlargements, the material was microscopically examined and found negative for spirochetes. Subcutaneous inoculations of 0.1 cc of material containing spirochetes were made into the same animals on September 20. These inoculations also failed to produce typical gangrenous lesions like those found in the pig; and upon microscopic examination, the material from these wounds was found negative for spirochetes and also for *Bacillus necrophorus*.

408

THERAPEUTIC AGENTS IN THE TREATMENT OF SCIRRHOUS CORD AND RHINOHYPERPLASIA

Therapeutic agents were tried in combating the spirochetes. It was believed to be necessary to use a drug having some specific action on this type of organism. As the infecting agent was not found inhabiting the blood stream, intravenous injections were not tried. Because of the location of the spirochetes, it was necessary also to have a drug which would penetrate into the tissue enough to attack the organism. Antimony and potassium tartrate (tartar emetic) seemed to have the desired qualities. Most arsenical preparations such as salversan, etc., used in human spirochetosis, would be too expensive.

The tumor-like growths were incised to allow free drainage, the necrotic, foul-smelling contents were removed, and powdered antimony and potassium tartrate was placed into the cavities. Enough of the drug should be used to coat over the inside area affected. If too much of the powder is placed into the cavity in the presence of a thin, fibrous capsule, absorption will be so great as to produce poisoning and death.

Antimony and potassium tartrate was used in an equeous solution and in glycerin, but the results were unsatisfactory.

Sodium cacodylate was tried with varying results; more experimental work will be necessary to prove its value as a therapeutic agent in these conditions.

Animals Treated with Antimony and Potassium Tartrate.—Pigs Nos. 11 and 12, both Poland China boars, weighing 40 pounds were inoculated on September 4, 1928, with 1 cc of material containing spirochetes from scirrhous cord of Pig No. 3. Material was placed into an abrasion in the nasal passages. The animals developed an enlargement approximately 4 cm in diameter that later ruptured and emitted a foul-smelling material, which on examination showed the presence of spirochetes. On September 20, 1928, both of these animals showed a characteristic rhinohyperplastic condition. The enlargement was incised, the foul-smelling necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavity. Four days later a considerable decrease in the size of the swelling was noticed; and on December 4, these animals showed very little deformity of the face and had apparently recovered from the infection, the pigs weighing about 150 pounds each. Pigs 61 and 62, both Poland China gilts weighing 65 pounds, had rhinohyperplasia. On December 12, 1928 the enlargements were incised, necrotic material was removed, and powdered antimony and potassium tartrate was placed in the cavities. The enlargements receded and the wounds healed, leaving just a small thickening over one side of the snout. The animals were discharged as cured on December 26.

Pigs 100 to 138, 38 animals in all, Duroc Jerseys, Poland Chinas, and Berkshires, had scirrhous cords approximately 2 to 6 cm in diameter, these growths having developed within the two weeks after castration. On December 21, 1928, the growths were incised, and after the necrotic material had been removed from the center, enough powdered antimony and potassium tartrate was then placed into the cavities to coat over the surface. There was considerable swelling within the next 12 hours, followed by an increase in the discharge from the wounds. One week later, the animals were examined, any loose necrotic material remaining was removed, and the cavities were washed with a mild antiseptic solution. Fourteen days following the placing of the powdered antimony and potassium tartrate in the cavities of these scirrhous cords, the animals were discharged as cured and sold on January 9, 1929.

SUMMARY

Spirochetes were present in all tissue specimens of scirrhous cord and rhinohyperplasia. The spirochetes were easily demonstrated in the discharge from these conditions when stained with carbol-fuchsin and Giemsa's stains. In the tissue preparations, stained by Levaditi's and Warthin's methods, the spirochetes were observed close to the line of necrosis in the hyperplastic tissue.

Bacillus subtilis and Staphylococcus pyogenes albus were also always present in the discharge, and a Gram-negative bacillus was found occasionally, which, when cultured, seemed to grow in symbiosis with Bacillus subtilis.

Bacillus necrophorus was isolated from three cases of rhinohyperplasia, and the organisms were associated in each case with spirochetes. As a result of the observations and experimental work, the conclusion seems justified that the presence of spirochetes in every case indicates that they are the etilogical factor in these hyperplastic growths, and the Bacillus necrophorus, a secondary invader. The spirochete has not been cultivated in pure culture. The spirochetes are located more deeply in the lesions than the other organisms, this fact suggesting their pathogenic influence, and etiological relationship in the production of scirrhous cord and rhinohyperplasia.

Scirrhous cord and rhinohyperplasia could not be experimentally reproduced by injection of the pure cultures or pooled cultures of organisms other than spirochetes isolated from these lesions. When, however, the mixed cultures containing the spirochetes were injected, the diseases were readily reproduced.

The inoculations of pure cultures of *Bacillus necrophorus* into healthy pigs, did not produce lesions similar to those obtained by the inoculation of tissue containing spirochetes.

Work done heretofore on the effect of light rays and restricted nutritional regimes on skeletal deformities of hogs has effectively eliminated the supposition that rhinohyperplasia is caused by or constitutes a complication of rachitis in swine.

Antimony and potassium tartrate (tartar emetic) was tried as a therapeutic agent, with about 90 per cent recoveries; but precautions were necessary to prevent poisoning from absorption because of too large amounts placed in the incised growths.

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The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

- 1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
- Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. B. C. Haas. April, 1923.
- Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
- 6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
- 7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
- Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
- Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
- The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
- 11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.
- 12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.
- Some Mutual Effects on Soil and Plant Induced by Added Solutes, by John S. Burd and J. C. Martin. December, 1923.
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- 20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Belationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.