STUDIES ON THE ETIOLOGY, PATHOLOGY, AND CONTROL OF GUINEA PIG LYMPHADENITIS

by

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INTRODUCTION

Lymphadenitis has been a serious disease of laboratory guinea pigs used in research for many years. The etiology of this disease has been reported as being a hemolytic streptococcus belonging to Lancefield group C, to which has been given many species names. Workers have previously reported no satisfactory treatment because of the nature of the organism, the localization of the disease process, and the sensitivity of guinea pigs to many antibiotics. Immunological studies have also met with failure in attempting to produce an artificially induced immunity to the infection. Recently an outbreak occurred in one of the guinea pig colonies on the campus of Kansas State University.

The object of this study was to examine the etiology, gross and microscopic pathology, and to attempt to control the disease through the use of sulfanilamide and an organic iodide, ethylenediamine dihydroiodide, in the feed.

It is essential to study more completely this disease and its control due to the greater emphasis being placed on disease-free laboratory animals for more exacting medical research.

REVIEW OF LITERATURE

History and Background

The disease was first reported in the United States by Boxmeyer in 1907. He described the disease as epizootic lymphadenitis or lumps. The condition was described accurately, and it resembles very closely the form which we see today.

Holman (1916) reported that streptococci-producing cervical abscesses and other lesions were one of the spontaneous diseases which occurred in the guinea pig.

Hardenberg (1926) reported on a survey of 750 guinea pigs in one colony which had 34 animals showing abscesses. He reported that 31 females and 3 males were affected. Twenty-four abscesses were found in the cervical region, 11 in the submaxillary area, 4 in the precervical region, 2 in the parotid area, and 2 in the inguinal region. Their general health did not seem to be impaired to any extent. The process was further described as being a "cold abscess" with no reaction to the surrounding tissue from the lymph glands.

Parsons and Hyde (1928) reported their observations of an epidemic of lymphadenitis in a complement-deficient guinea pig colony. During the five-year observation period, lumps frequently appeared. A streptococcus was always isolated. Cunningham (1929) reported on a colony of 150 guinea pigs where he found 20 to be infected--15 females and 5 males. Of the 20 abscesses found, 4 were in the cervical region, 10 submental, 2 submental-cervical, 1 retroperitoneal, and 2 retroperitonealinguinal. One animal had a caseous pneumonia.

Megrail and Hoyt (1929) reported on an outbreak of the disease in a research colony. They found of 35 abscesses: 27 in the cervical region, 5 in the neck region, and 3 in the cheek region.

Beattie (1931) described an outbreak starting in the middle of June in 1927 and continuing until December of 1928, with no

more cases appearing subsequently. Sixteen cases were observed: 11 from stock not used for experimental purposes and 5 which had been injected with material from patients with suspected tuberculosis which were later proved negative. He observed the abscesses most commonly in the cervical region.

Smith (1941) reported on guinea pigs which exhibited swellings of the lymph glands which contained caseous pus, from the medical research council's farm laboratory. He noted in some there was retrogression of the lesions with apparent complete recovery, and in others a continuous enlargement of the abscess until it finally broke open and discharged pus. He reported the etiology to be an organism differing from the etiology which most authors have reported.

Hartwigk (1949), in Germany, reported on a suppurative inflammation of the lymph glands of guinea pigs. He called the condition "lymphadenitis purulenta aposthematosa." This condition was a spontaneous disease of the guinea pig and was a confusing disease when associated with animals used for the diagnostic test for tuberculosis.

Torres et al. (1949) reported on this disease from Brazil, finding it in a group of guinea pigs imported from the United States. Nodules were observed on palpation, and found occasionally to ulcerate and discharge pus. They found that some animals survived with regression of the lesions. Others died with an acute fibrinopurulent peritonitis and multiple liver abscesses.

Imaizumi et al. (1955) reported on 10,000 guinea pigs which were shipped to Japan. A large number arrived dead, and many died after arriving. Later the disease appeared among colonies in several districts of Japan. It is not clear if the original outbreak was blamed, or if the disease was already present, although latent.

Disease Process

The disease is manifested most often as a chronic localized abscess-forming condition (Boxmeyer, 1907; Parsons and Hyde, 1928). The lymph glands affected are most often those of the head and neck region (Cunningham, 1929; Megrail and Hoyt, 1929). Parsons and Hyde (1929) found the abscesses were first palpatable when about the size of a pea, and were very firm. The nodes gradually increase in size, soften, and may continue to enlarge until they are the size of a walnut. The lesions usually broke open and drained to the outside. After draining, the abscess may regress and disappear. It was found that surgically incising the abscesses would give the same results. They observed that the general health of the animal was not impaired and that death rarely resulted from this type of infection. The greatest number showing the infection were between 12 and 15 months of age. Boxmeyer (1907), Torres (1949), and others have reported a systemic form which kills the guinea pigs rather rapidly.

Transmission

Boxmeyer (1907) reported that the disease was transmitted "without a doubt" by a wound infection spread by pus from the ruptured abscesses. He felt the organism entered through abraided mucous membranes of the throat which was caused by ingesting rough food. Further experimentation along these lines was accomplished by feeding whole oats contaminated with cultures of the organism to susceptible guinea pigs. All showed temporary swellings of the cervical lymph glands, and one developed a typical abscess. Boxmeyer also explained the involvement of the auricular glands by the biting habits of guinea pigs.

The disease has been transmitted experimentally by several different methods. Parsons and Hyde (1928) injected broth culture and pus emulsion in separate experiments in 0.25 cc amounts intraperitoneally. All animals died within 48 hours. They further experimented with transmission of the disease by feeding guinea pigs 2 cc of 24-hour broth culture with a pipette. Their results were somewhat inconclusive although a few did develop swellings apparently of the lymph glands which subsequently regressed. A very few developed abscesses from which a hemolytic streptococcus was isolated.

Megrail and Hoyt (1929) fed cultures of hemolytic streptococci and staphylococci to guinea pigs with no effect. They further rubbed a culture of the streptococci vigorously on the oral mucosa with no conclusive results. Chopped dry hay seeded with the streptococcus culture was fed, and the feeding repeated 36

hours later with only one animal developing an abscess. Four guinea pigs were exposed to aersol spray of the streptococcus. One died in six days with a streptococcal pneumonia. The other three were still normal in 60 days. Megrail and Hoyt further inoculated guinea pigs by scratching the skin of the neck and groin regions with a needle and then seeding the area with culture. They were successful in reproducing the disease in this manner.

Hardenbergh (1926) further attempted experimental transmission by the conjunctival route. This method failed. He also placed healthy animals with guinea pigs which had infected, draining abscesses. After two months, the healthy animals were still apparently normal. Further work by Hardenbergh proved that no toxin is produced either by broth culture or from one which is detrimental to guinea pigs.

Seastrone (1939) found by injecting 10^{-2} dilution of culture intradermally that he could produce the disease syndrome very similar to that seen in the natural chronic infection. He further worked experimentally to prove natural transmission of the disease by separating infected and healthy animals by 2½-inch screening 1 inch apart. Healthy guinea pigs were placed in with the infected animals as controls. The guinea pigs which were separated by 1 inch of space and screening failed to become infected while the controls in with the infected guinea pigs became infected. He exposed animals to aersol infection and seeded oats with culture. Four animals of six died of extensive pneumonia from

the aersol infection and the other two developed deep-seated abscesses of the bronchial lymph nodes. The eight guinea pigs exposed to culture-seeded oats all developed enlarged cervical lymph nodes typical of natural infection. He therefore concluded that in nature the disease is spread by ingestion of the organism.

Etiology

Most of the authors, with few exceptions, are in agreement that the etiology of the disease process is a hemolytic streptococcus. Boxmeyer (1907), in his early description of the disease, mentioned a coccus as the etiology. It was usually seen as a diplococcus or in short chains of four to six organisms. Many of the early authors described the organism as being _Streptococcus pyogenes_ (Beattie, 1931; Hardenbergh, 1926; Holman, 1916). All authors except Smith (1941) agreed that the etiology was a hemolytic streptococci. Smith (1941), in his work, found an organism which was _Streptobacillus moniliformis_. Authors writing after Lancefield (1933), with the exception of Smith (1941), found the organism to be Lancefield type C by serological classification (Moen, 1936; Foley, 1944; Hartwigk, 1949; Torres et al., 1949). Imaizuma (1955) reported the organism to be _Streptococcus hemolyticus_. Breed et al. (1957) reported _Streptococcus zooepidemicus_ as the etiology of guinea pig lymphadenitis.

Jennings (1928) reported a dissociation of the streptococcus which causes this disease and which he called _Streptococcus hemolyticus_.

Immunology

Herbert and Meyer (1929) reported on cutaneous immunization of guinea pigs against this disease. They prepared a Besredka filtrate by growing the organism in veal infusion broth with 0.1 per cent glucose for 10 days. This material was then filtered through a mandler filter. This filtered material was then re-inoculated and found incapable of supporting further growth. The organisms were then filtered out, and the filtrate used for the antigen. Guinea pigs were given the filtrate in dosages of 1 cc to 2.5 cc. Subsequent challenges revealed no protection at all. Vaccines were then prepared by growing the organism in broth, centrifuging and resuspending the organisms in 0.85 per cent salt solution. It was then killed by heating at 56° C. for one hour. Single doses of the vaccines resulted in no immunity as a response to challenge. Likewise no protection was evident from three injections of the vaccine. They additionally injected sterile broth intradermally to attempt a non-specific local resistance. This also was unsuccessful.

Seastrone (1939) prepared heat- and formalin-killed vaccines of the organism. He also prepared aggressins by injecting guinea pigs intraperitoneally with virulent culture. The guinea pigs were killed in two days, and the viscid material in the peritoneum was washed out and treated by heating. All vaccination results failed as the animals all succumbed to the challenge infection. Similar trials with mice also met with failure. Experiments were also attempted to produce immunity by injecting guinea pigs with

serum from both chronically infected guinea pigs and immune serum produced from rabbits. Subsequent challenge proved infectious with no resistance seen. Similar results were observed when tried in mice. He further studied immunity in the presence of chronic infection. Guinea pigs were infected by injecting culture intradermally in the flank. After the infection was established, the guinea pigs were inoculated intradermally in the opposite flank. He concluded from this experiment that some resistance to superinfection was found in the guinea pigs carrying natural abscesses. He also observed that no opsonizing antibody for young virulent cultures could be detected.

Foley (1944) attempted to determine if it would be possible by an allergic inflammatory response per se to localize bacteria given as a reinfecting dose. Four groups of guinea pigs were utilized. Group I was given several injections of formalized culture. Challenge eight days later revealed no immunity either general or local. In group II were animals with the infection of six-days duration which were given reinfecting injection of the live organism. This also resulted in no local or generalized protection. Group III animals had been chronically infected for three-weeks duration. Reinfection revealed no local immunity; however, the infection did not extend to the regional lymph nodes. This research, in general, revealed no significant immunity being produced either by artificial vaccines or chronic or acute predisposing infections.

Moen (1936) found that guinea pigs infected with this hemolytic streptococcus would react to a crude bacterial extract when given intradermally. The reaction of the skin test reached a peak in 24 hours. Animals would react as soon as five days following infection. Although the extract was crudely prepared, results were very accurate. Some animals were detected which were infected with the organism, but did not show external lesions.

It was therefore shown that little or no protection was derived through the use of artificially prepared vaccines or specific antisera, or from chronic present infection.

Gross Pathology

Gross pathology was generally confined to enlargement of the lymph glands of the head and neck region with subsequent formation of abscesses within these glands. It was also noted that other lymph glands may be involved but to a much lesser extent (Boxmeyer, 1907; Hardenbergh, 1926; Parsons and Hyde, 1929; Cunningham, 1929; Megrail and Hoyt, 1929; Beattie, 1931; Hartwigk, 1949). Abscesses range from the size of a pea to the size of a walnut (Parsons and Hyde, 1929). Beattie (1931) described the size as 1 cm in diameter to 4 cm in diameter.

Boxmeyer (1907) mentioned there may be from 1 to 10 abscesses present in one animal.

Hardenbergh (1926) related the formation of a "cold abscess" with no inflammatory reaction in the surrounding tissue.

Histopathology

Boxmeyer (1907) reported that in the early stages of the disease, lymph nodes undergo a marked dilation of the lymph sinuses and a desquamation of their endothelial cells. Cells are ragged in outline and show degenerative changes. The nuclei have little chromatin, and the cytoplasm stained faintly pink with eosin. These cells tend to collect in the cortical sinuses where they often fuse to form multinucleated masses of considerable size. The germ centers show marked activity although a decrease in the number of lymphocytes is observed. The capsule shows local proliferation, and in these places the peripheral lymph sinus is obliterated and fibroblasts invade the cortex. Endothelial cells and fibroblasts form dense masses of large multinuclear cell masses and their marked increase in size enlarge the lymph node. In later stages of the disease, polymorphonuclear leucocytes appear in the center of the cell collections and may be taken up by mononuclear cells. Lymph nodes increase in size due to the increased number of mononuclear and polymorphonuclear cell increases. Foci soften in the center, and pus is formed like ordinary abscesses. Fibroblasts form a thicker capsule and become a true pyogenic membrane.

Beattie (1931) described similar lymph node lesions, adding that in the foci of degeneration in the nodes, large numbers of streptococci are seen. He also mentioned that the nodes show the center of the foci to be occupied by coagulated necrotic material with no nuclei being stained. This area is surrounded by a dense

ring of polymorphonuclear leucocytes and beyond that, proliferating fibroblasts. Beattie further reported on the histopathology of affected lungs. A patchy pneumonia was seen with a very marked granulation tissue formation which encloses neutrophiles in the alveolar walls, surrounding bronchi, and blood vessels. The original lung structure may be obliterated. Scattered throughout the lung were various-sized areas of coagulation necrosis surrounded by neutrophiles as seen in the lymph node lesions.

Torres et al. (1949) described the microscopic lesions of the disease as an acute fibrinopurulent peritonitis, acute suppurative lymphadenitis, and necrosis and abscessation of the liver. They found clumps of bacteria in the lumina of the lobular vessels and in the adjacent sinusoids of the liver. The whole lobule showed coagulation necrosis. The edges of the necrotic areas were lined by an infiltration of heterophilic leucocytes. In only a small part nearest the portal spaces were there healthy liver cells. Their description of the microscopic changes of the lymph glands follows closely to previous descriptions. These workers further explained that the acute inflammatory processes found in the lymph glands, peritoneum, and liver had the usual characteristic of this type of inflammation. There is considerable proliferation of fibroblasts in the tissues around the inflammatory process. It is of note, however, its exaggerated form in the guinea pig as compared to other animals and man.

Hartwigk (1949) described the lesions as purulent exudation with fusion of leucocytes, infiltration of lymphatic tissue, and a closely formed connective tissue capsule.

Treatment

Karel et al. (1941) studied the course of infection and effectiveness of sulfanilamide in relation to the vitamin C content of the guinea pig. It was found that without sulfanilamide treatment, the course of infection as measured by the survival time, was not influenced by either a high ascorbic acid level or by a 10-days vitamin C depletion. Treatment with sulfanilamide was effective, but neither high ascorbic acid content nor 10-days vitamin C depletion influenced the effect of the drug.

Jones (1957) told of the use of sulfanilamide as a bacteriostat in the treatment of streptococcal infections in general.

Smith (1956) reported on the use of ethylenediamine dihydroiodide (Hi-Amine) in the treatment of jowl abscesses in swine caused by a streptococcus. It was administered orally in the feed or water.

Hematology

Hardenbergh (1926) mentioned that in his experience with the disease, the blood picture was variable and showed no significant change.

Bilbey and Nicol (1955) reported on the normal blood picture of 53 normal male guinea pigs weighing 300 to 570 grams.

Red blood cell counts revealed 5 million per cubic mm with a variation of 0.4 million. The average total white cell count was 9,100 cells per cubic mm which varied from 5,000 to 15,000 with most counts between 8,000 and 10,000. The differential count was reported as follows: Lymphocytes, average 64 per cent, with a range of 55 to 81 per cent; psuedoeosinophilic granulocytes, average 32 per cent with a range of 16 to 44 per cent; immature granulocytes, average 0.1 per cent with a range of 0 to 1 per cent; eosinophilic granulocytes, average 0.6 per cent with a range of 0 to 3 per cent; basophilic granulocytes, average 0.2 per cent with a range of 0 to 2 per cent; monocytes, average 3.1 per cent with a range of 0 to 7 per cent. The psuedoeosinophilic granulocyte is the counterpart of neutrophiles of human blood. When compared with true eosinophiles, they show finer granules in the cytoplasm, do not exhibit certain specific staining, and show multiple segmentation of the nucleus from four to seven lobes.

Control

Parsons and Hyde (1928) outlined steps on controlling the infection in a guinea pig colony. The first step is to isolate all affected animals. If an abortion occurs, remove the female. Regularly palpate the guinea pigs for lumps. If found, isolate the animals, even if the lumps later disappear. Keep isolated animals in a separate room.

Imaizumi et al. (1955) described control measures used in Japan to ascertain clean stock for the medical laboratories.

All breeders furnishing guinea pigs to the laboratories were registered, and then animals so marked. If any of the guinea pigs were infected they could be traced back to the breeder and district involved.

DeSomer et al. (1955) reported on the toxicity of penicillin when used in guinea pigs. They explained that the normal intestinal flora of the guinea pig is primarily gram-positive organisms. All of these aerobic gram-positive bacteria except enterococci are sensitive to penicillin. After using penicillin, the animals sicken and die as the intestinal flora changes to predominantly gram-negative organisms, belonging to the coliform group. These bacteria produce a toxin which is fatal to the guinea pig. Guinea pigs whose intestinal flora was constantly exposed to a low level of penicillin showed after four weeks a typical syndrome of illness resembling a state of deficiency or chronic intoxication. A dose of 5,000 units intraperitoneally caused 60 to 70 per cent death. One hundred thousand units intraperitoneally caused 90 per cent death.

Stevens and Gray (1953) found 70 per cent mortality with penicillin a dose of 20,000 units of the potassium salt. They further found 93 per cent mortality with 20,000 units of the procaine salt. Eyssen et al. (1957) reported mortality in guinea pigs from the use of penicillin, bacitracin, and chlorotetracycline and streptomycin. Chloramphenicol did not cause death.

Serology

Cunningham (1929) reported agglutinins were present for the organism from infected guinea pigs.

MATERIAL AND METHODS

Experimental Animals

Animals utilized for histopathological studies were guinea pigs of both sexes obtained from the colony of the Department of Pathology, Kansas State University. They weighed between 300 and 450 grams and were approximately four months of age. The feed consisted of Laboratory Chow pellets[1] plus green lettuce leaves. They were further observed two weeks prior to inoculation and carefully examined and palpated prior to the experiment. They were divided into three groups: groups I, II, and III.

Guinea pigs used for the feeding trials were obtained both from a commercial firm[2] and from the department's colony. They were handled as described for the above groups except that the pellets were ground in order to mix the medication administered. It was necessary to calculate amounts of feed consumed accurately; therefore, special procedures were necessary to prevent the animals from throwing and kicking the feed out of the feeding bowls. Screening with a 7/16-inch mesh was used to form four compartments in each feeding bowl, and protruded above the bowl

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[1] Purina Feed Co., Davenport, Iowa.

[2] National Laboratory Animal Co., Creve Coeur 41, Missouri.

approximately two inches. This effectively prevented the guinea pigs from wasting any of the medicated ground feed. The two-week observation period allowed the animals adequate time to become accustomed to the feed. The amount of feed consumed during this two-week period was measured daily by volume. Additional feed was then added each day.

Inoculation, Collection, and Preparation of Material for Gross Pathology and Histopathology

Guinea pigs in groups I, II, and III from the department's colony were inoculated by three different routes. Group I was given 0.4 cc of culture subcutaneously. Group II was given 0.2 cc of culture intradermally. Group III was shaved on the right side of the neck. The area was then disinfected with alcoholic Roccal[3] and thoroughly dried with sterile gauze. A sterile 20-gauge needle was used to perforate the skin in several places. Attempts were made not to penetrate the subcutis. Sterile cotton swabs were soaked in the bacterial broth culture and rubbed vigorously over the area.

The culture used was a hemolytic streptococcus which was isolated 48 hours earlier from an abscessed lymph node of a naturally infected guinea pig. The organisms were removed from the 5 per cent sheep blood agar plate and transferred into 5 cc of sterile trypticase soy broth[4] 24 hours prior to injection.

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[3] Winthrop Laboratories, New York 18, New York.

[4] Baltimore Biologics Laboratory, Baltimore, Maryland.

Four animals were inoculated in each group; in three days the lymph nodes in all animals in the three groups were palpatable.

One animal from each group was necropsied on the third, tenth, sixteenth, and thirtieth day postinjection. This procedure was followed in all groups except group I, in which an early death loss in the experiment occurred and the sixteenth-day necropsy was omitted.

The animals were euthanized by chloroform inhalation with the guinea pig being placed in a bell jar. A complete necropsy was then performed and gross lesions were noted. Affected lymph nodes; lung, heart, spleen, kidney, and liver specimens were taken for bacteriological study. Each organ was cultured on 5 per cent sheep blood agar and a small portion placed in 5 cc of sterile trypticase soy broth. If no growth was evident in 24 hours on the agar plates, the broth culture of that particular organ was then streaked on a 5 per cent sheep blood agar plate. Another portion of the organ, at the time of necropsy, was immediately placed in 10 per cent buffered formalin in a volume of at least 10 times the volume of the tissues. The tissues fixed in the formalin solution were trimmed 48 hours from the date of collection. Sections of the tissues were prepared by the paraffin method. Several slides of each tissue were prepared. Sections were stained with hematoxylin and eosin, trichrome, Giemsa, and Gram's stains.

The same procedures as described for the three groups were employed cn a normal, healthy 350-gram guinea pig to obtain normal sections for comparison.

Feeding Trials

Seven groups of guinea pigs were used for the feeding trials. Groups A, B, C, D, and E were guinea pigs obtained from a commercial firm. Groups F and G were guinea pigs from the department's animal colony. Each group consisted of 10 guinea pigs. There was no attempt to segregate animals on the basis of sex. Most of the guinea pigs were white or light in color which allowed potassium permanganate to be used as a dye to identify the guinea pig in each group. Those which did not lend themselves for dye marking were identified by their color pattern.

The groups were clipped on the right side of the neck, using an Oster clipper[5] with a size 40 surgical blade. The area was disinfected with alcoholic Roccal and then thoroughly dried with sterile gauze. A 20-gauge needle was used to perforate the skin in numerous places. Groups A, B, C, and D were injected with an 18-hour broth culture of the hemolytic streptococcus which was isolated 48 hours previously from an infected guinea pig. Groups E. F, and G which served as controls were swabbed with sterile broth, the same type of medium in which the organism was grown for groups I, II, and III.

Blood specimens were obtained from three guinea pigs from each group for a total of 21 animals prior to infection to establish normal red blood cell count, white blood cell counts, and white blood cell differential counts. Blood samples were collected by clipping the long hairs around one of the toenails on

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[5] John Oster Mfg. Co., Milwaukee, Wisconsin.

one of the rear feet. The nail and surrounding area was then disinfected with alcoholic Roccal and allowed to dry. A pair of surgical scissors was then used to clip the end of the toenail down to the bleeding tissue. When a drop of blood appeared, the 20 lambda coulter pipette[6] was used to withdraw exactly 0.02 ml of blood. This was added immediately to 10 cc of triple-filtered 0.85 per cent saline solution. Blood smears were made directly from the toe, and the slide air dried. The foot was then grasped tightly to restrict the flow of blood, and a silver nitrate applicator stick applied to the bleeding surface to cauterize the area. When the blood ceased to flow, the animal was replaced in the cage. Blood smears were fixed with methyl alcohol and stained with Giemsa stain.

One hundred lambda coulter pipettes were utilized to draw 0.1 ml of the original 10 cc of the blood saline mixture and further diluted into an additional 10 cc of 0.85 per cent saline solution. This dilution was utilized for the red blood cell count and was a 1:50,000 dilution. The dilution for the white blood cell counts was 1:500. The red cell counts were made with the Coulter Electronic Cell Counter.[7] Prior to counting cells, the background count of particles inherent to the filtered saline was made at the threshold settings and aperature setting used for both the red blood cells and the white blood cells. Duplicate counts were made and the average count was designated as the background count.

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[6] Coulter Electronic Sales Co., Hialeah, Florida.

[7] Ibid.

Previous experience revealed the correct settings to be an aperture current of 5 and a threshold setting of 7 for the red cell counts. After counting the red cells, 0.1 cc of a 1:10,000 saponin solution was added to each 10 cc of the 1:500 dilution for the white blood cell counts. This was necessary to lyse the red blood cells present. When the solution appeared as a clear, uniform, bronze color, the white blood cells were counted. The aperture current used for the white cell counts was 5 and the threshold setting was 17.

Duplicate counts were made on each blood sample, the average calculated, the background count subtracted, and then the actual count calculated from the count loss correction chart which accompanies the Coulter Counter. This then was the corrected cell count. The stained blood smears were examined under oil immersion, and 100 cells were counted from each smear.

The blood samples were obtained from the guinea pig from each group in numerical order daily for 15 days. Samples were then drawn every other day for the next 15 days.

The standard ground feed was fed to all animals following the scarification and inoculation for three days until 100 per cent of the animals were infected as ascertained by palpation and close examination. At this time the animals were placed on the medicated feed. All medicants were weighed to the nearest 0.01 gram and mixed for 15 minutes with the ground feed in a Hobart bakery mixer.[8]

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[8] The Hobart Co., Troy, Ohio.

The animals were fed the medicated feed at a dosage schedule per pound of body weight as determined by averaging the body weights of each group of animals. The groups were reweighed at 15 days to recalculate the medicated feed dosages for the remainder of the 30-day feeding experiment.

Group A: Animals were fed the salt base ethylenediamine dihydroiodide[9] at a rate of 2 grains per 20 pounds of body weight as suggested by Burch (1962).

Group B: Animals were fed sulfanilamide[10] at a rate of 0.1 mg per pound at a feeding schedule of four days on the sulfa feed and seven days off the feed for the first 15 days. A dosage rate of 1 mg per pound was then used for the next 15 days with a feeding schedule of four days on the sulfa and seven days off.

Group C: Animals were fed both the ethylenediamine dihydroiodide and sulfanilamide at the exact dosage rates and schedules as in groups A and B.

Group D: Animals were infected controls and were not given any medicated feed.

Group E: Animals were non-infected controls with no medication.

Group F: Animals were given sulfanilamide in the feed at the dosage schedule of group B--these to serve as non-infected sulfa controls.

Group G: Animals were given ethylenediamine dihydroiodide

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[9] (Hi-Amine) Pitman-Moore Co., Indianapolis, Indiana.

[10] Sulfanilamide Powder, Curts-Folse Laboratories, Kansas City, Kansas.

at the dosage schedule of group A to serve as non-infected organic iodine controls.

At the conclusion of the 30-day feeding period, all animals of the groups A, B, C, and D were euthanized by injecting Nembutal[11] at a rate of 1 cc per guinea pig intraperitoneally. Groups E, F, and G were all examined and palpated, and found to be free from any glandular swellings.

Necropsies were performed on all animals from groups A, B, C, and D. All enlarged lymph nodes or if not grossly enlarged, regional lymph nodes of the head and neck, liver, lung, heart, blood, spleen, and kidney were inoculated on 5 per cent sheep blood agar. In addition, following streaking, the tissues were placed into 5 cc of trypticase soy broth and if no growth ensued from the original agar plates in 24 hours, new plates were streaked from the broth tubes. The blood agar plates were observed at 24 and 48 hours for evidence of hemolytic streptococci. Positive and negative results were recorded.

The above animals were recorded as having or not having the characteristic enlarged lymph nodes. If the lymph nodes were enlarged, two dimension measurements were made in centimeters.

Bacteriology

Four isolates from four different guinea pigs, all showing natural infection, were studied. The pus was collected by aspirating from the abscess, using a sterile glass syringe and

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[11] Abbott Lab., North Chicago, Illinois.

needle. The pus was then streaked on 5 per cent sheep blood agar and allowed to grow for 18 hours. Colonies from each plate were placed in trypticase soy broth, each one maintaining its identity. All of the test media were inoculated from this material, using a sterile pipette and placing 0.25 cc of broth culture in each tube of the liquid test substances. Solid media plates were inoculated by streaking with a wire loop.

The following biochemical tests were utilized in determining the etiology of the organism.

1. Catalase test
2. Solubility in bile
3. Growth in 10 per cent bile
4. Growth in 40 per cent bile
5. Growth in 6 per cent NaCl
6. Growth in 2 per cent NaCl
7. Reduction of nitrates
8. Liquefaction of gelatin
9. Growth in 1 per cent methylene blue milk
10. Growth in litmus milk
11. Growth in 5 per cent sucrose
12. Growth in pH 9.6 broth
13. Hydrolysis of Sodium Hippurate
14. Hydrolysis of starch
15. Splitting of esculin
16. Carbohydrate fermentation:
    a. Lactose
    b. Sucrose
    c. Trehalose
    d. Sorbitol
    e. Salacin
    f. Raffinose
    g. Mannitol
    h. Xylose
    i. Galactose
    j. Rhamnose
    k. Dextrose
    l. Glycerol
    m. Maltose

The sugar-free medium used for the carbohydrate fermentation reactions was composed of the following ingredients as suggested by Foltz (1962):

Proteose Peptone #3	20 grams
Salt	7.5 grams
Beef Extract	5.0 grams
Distilled Water	1,000 cc

The pH was adjusted to 7.6, and brom thymol blue was used as the indicator.

The determination of the optimum growth temperature was accomplished by melting glucose yeast extract agar with added beef extract and holding the medium at 45° C. This medium was inoculated by stabbing a straight wire loop which had previously been seeded with culture. The tubes were immediately plunged into ice water and allowed to harden. Three tubes were prepared from each of the four unknown organisms and three tubes of each of the known test organisms. One tube from each organism was placed in a 10° C. incubator, one each in a 37° C. incubator, and one each in a 45° C. incubator. The tubes were observed daily for seven days for evidence of growth.

The heat tolerance test was accomplished by adding organisms from a 24-hour blood agar plate to 1 cc of sterile saline in a tube, and heating for 30 minutes in a 60° C. water bath. This material was then added aseptically to 5 cc of trypticase soy broth and incubated for seven days at 37° C. and observed daily. The unknown organisms and the known test organisms were tested simultaneously.

All four unknown isolates were Lancefield typed, using the Lancefield typing reaction with methods and typing sera of the

Difco Company.[12] The autoclave method of preparing the antigen was used. All antigens were tested against known sera, types A, B, and C.

The fibrinolytic test was performed by diluting 0.2 ml of oxalated human plasma with 0.8 ml of saline, and 0.5 ml of an 18-hour broth culture of the unknown organism was added. To this was added 0.25 ml of 0.25 per cent aqueous solution of calcium chloride and mixed. The mixture was placed in a water bath at 37° C. In about 10 minutes a solid coagulum was formed. The tube was then observed every 10 minutes for liquefaction.

The organisms were examined for the presence of a capsule. Cultures were grown in trypticase soy broth and observed at 6-, 18-, and 24-hour intervals. Additional cultures were grown in trypticase soy broth with added sterile guinea pig serum and observed at 6, 18, and 24 hours. The previously mentioned cultures were stained with India ink alone, India ink counter-stained with carbol fuchsin, Hiss' capsule stain, and Anthony's capsule stain.

All biochemical media were inoculated simultaneously with each of two test organisms--one organism which was known to produce a desired positive reaction in the medium and one which was known to produce a negative reaction. The test organisms were obtained from the Department of Bacteriology at Kansas State University, and enough biochemical reactions were used on each particular organism to verify its identity. Blood agar plates were inoculated using the following species of blood in a 5 per cent concentration: horse, ox, man, rabbit, and guinea pig.

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[12] Difco Labs., Detroit, Michigan.

These plates were observed at 24 and 48 hours, and results and observations were recorded.

Biochemical reactions were noted as positive reactions when they occurred, and if no reaction occurred they were incubated for a total of seven days. The positive reactions were verified by inoculating the media on 5 per cent sheep blood agar plates. Observations were then made for the typical hemolytic mucoid colony. After seven days, if the reaction remained negative, the tubes were observed for growth and streaked on the blood agar plates as was accomplished with the positive reactions.

Four mature white mice and four immature white rats were inoculated intraperitoneally with 0.25 cc of an 18-hour broth culture of the organism. One white rabbit was given 0.5 cc of an 18-hour broth culture of the organism intravenously. All animals were observed at 18, 24, and 36 hours.

RESULTS AND DISCUSSION

Gross Pathology

Group I, which had been injected with 0.5 cc of culture subcutaneously, developed swollen adjacent lymph nodes three days later.

Group II, which had been injected with 0.2 cc of culture intradermally, showed swelling of the adjacent lymph nodes and an intense reddening and swelling of the skin three days later.

Group III, which had been scarified, revealed swelling of the adjacent lymph nodes in three days. Very little skin reaction was observed.

Necropsies performed on the third day postinjection, revealed the following gross lesions:

Group I: A large nodular mass was found located between the mandible and just forward of the thoracic inlet. The mass was circular and was approximately 2.5 cm in diameter. The mass felt firm and was yellowish-grey in color. Upon incising the mass, a reddish serous fluid was observed. The cut surface of the mass appeared yellowish-grey with a considerable amount of edema present. Edema was also present in the tissue around the mass. The mass was identified as a regional lymph node. All other organs appeared apparently normal. A typical hemolytic mucoid streptococcus was isolated from the lymph node, heart blood, lung, spleen, liver, and kidney.

Group II: A nodular mass was found on the right side of the neck adjacent to the site of injection. Due to the large amount of edema and congestion around the node, it was difficult to ascertain its approximate size, which was about 1 cm in diameter. The cut surface was grey in color, and edematous. No other lesions were observed. Typical hemolytic mucoid streptococci were isolated only from the lymph node and liver.

Group III: A small firm nodule was found at the site of scarification near the right ear. Edema was extensive around the injection site and lymph node. The node measures 0.75 cm in diameter. All other organs appeared normal. The typical organism was isolated only from the involved lymph node.

Necropsies performed on the tenth day postinjection revealed the following findings:

Group I: A large nodule, 3 cm in diameter was found in the right cervical area near the right ear. The nodule was adhered to the skin and was soft. When incised, it was found to contain a very liquid yellow pus and a greyish solid area. All other organs appeared normal. The typical organism was isolated from the infected lymph node only.

Group II: A 3-cm area of the skin of the neck was indurated and reddened. Closely adherent to the skin was a 1.5 cm diameter firm nodule. The cut surface was grey with small yellow foci 0.1 cm in diameter, scattered throughout the node. Pus was not present. All other organs appeared normal. The typical organism was isolated from the infected lymph node only.

Group III: Two firm nodules were observed in the right cervical area, one 2.5 cm in diameter and one 1 cm in diameter (Plate I). Both were firm and grey as seen on the cut surface. All other organs appeared normal. The typical organism was isolated from the infected lymph node only.

Necropsies performed on the 16th day postinjection revealed the following gross lesions:

Group I: Due to an early death loss no animals were examined.

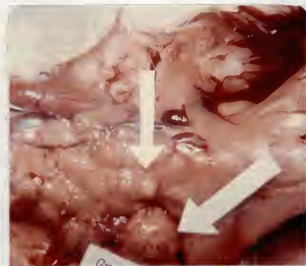
Group II: One nodule was seen in the intermandibular space. The nodule was 1 cm in diameter and contained thick creamy pus. All other organs appeared normal. The typical organism was isolated from the abscessed lymph node. Other tissues were negative on culture.

Group III: Three nodules were observed in the ventral region of the cervical area. Two nodules were 1.5 cm in diameter

EXPLANATION OF PLATE I

Cervical lymph nodes of an infected guinea pig (group III, scarification) 10-day postinjection, showing the gross enlargement, with no inflammation of the surrounding tissue.

PLATE I



and one nodule 1 cm in diameter. All three lesions contained thick creamy pus. The other organs appeared normal. The typical organism was isolated from the involved lymph node only.

The foregoing descriptions reveal a condition which appears first as an enlargement of the node with marked edema. As the disease progresses, pus pockets form in the tissue; finally the whole node becomes a large abscess.

It is of interest to note that on the third day postinjection, the organism was isolated from several different organs, whereas from the tenth day on, the organism apparently was confined to the lymph nodes nearest the injection site.

Histopathology

The histopathology of group III will be described in detail, and the other two groups will be briefly compared to group III.

Group III: Scarification, 3rd day postinjection

A. Capsule and periglandular tissue

1. Hyperemia of the area is noted, and edema is in evidence especially of the more external portion of the capsule. A large number of neutrophiles and an occasional eosinophile are noted in the capsular area surrounding the inner lining of the capsule. Exudation is evidenced by the fibrin, red blood cells, and white blood cells present throughout the capsule.

B. Lymph node

1. There is evidence of a marked lymphoid depletion. The germinal centers are primarily affected with a large number involved. Only a thin layer of normal lymphocytes surround them. The medullary portion of the node also shows marked depletion of lymphocytes. The lymph-cell depleted areas are replaced by large mononuclear cells and granulocytes, primarily neutrophiles. The gross enlargement is probably due to a large increase in the mononuclear epitheloid cells, especially in the area of the sinusoids. The streptococci are present in moderate numbers under the capsule and in large numbers in the lighter staining areas which are areas more depleted of lymphocytes. The organisms are also found scattered throughout the more normal areas in fewer numbers. They appear mostly as short chains of from three to five organisms with a few up to ten organisms in length, where they are found in greater numbers. In areas where there are fewer organisms, they are found in singles and pairs. The majority do not appear to be phagocytized.

The tissue section of the node could be summarized by saying the infection results in an acute lymphadenitis and periglandular inflammation, characterized by a diffuse heterophilic infiltration, primarily neutrophiles, with a few eosinophiles

present as well. Reticulo-endothelial cell proliferation and lymph cell depletion are also very much in evidence (Plate II).

Group III: Scarification, 10th day postinjection.

A. Capsule and periglandular tissue

1. The major change observed in this section is the proliferation of the capsule by fibroblasts with some mature connective tissue being laid down. In some areas, a few collagen fibers are extending down into the cortex of the gland. There appears to be a marked reduction in the number of inflammatory cells, but the neutrophiles, macrophages, and lymphocytes appear to be there in equal numbers. There is an increase in vascularity over normal and a definite angioblastic proliferation. All are indicative of early granulation tissue formation.

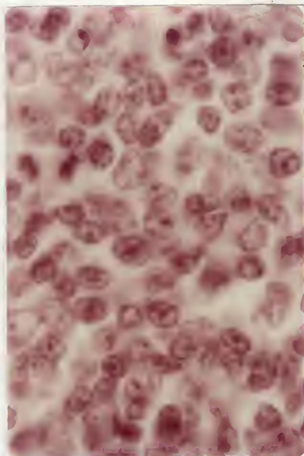
B. Lymph node

1. The cortex of the node seems to be primarily affected, especially the germinal centers. These centers appear as poorly circumscribed, coalescing inflammatory nodules that blend into one another. The nodules characteristically consist of a central, dense, cellular core and an outer exudative and proliferative inflammatory area. The necrotic core consists of a large amount of cellular debris, dead and dying neutrophiles, degenerating lymphocytes, and a few macrophages. Many of the bacteria

EXPLANATION OF PLATE II

Area of the cortex of a cervical lymph node of an infected guinea pig (group III, scarification) 3-days postinjection. There is evidence of a marked lymphoid depletion with these cells being replaced by large mononuclear cells and heterophiles. 1250X.

PLATE II



in this area appear to be phagocytized by the macrophages. The degenerating cells are undergoing pyknosis and karyorrhexis. The outer area of the nodule consists primarily of large mononuclear epitheloid cells. Neutrophiles are present in this area as well. Towards the outer margin of the nodule, a few lymphocytes appear. The margin of the nodule blends in with the surrounding normal lymphatic tissue. No distinct zone of margination as yet has appeared. The organisms are found less numerous under the capsule and more numerous in the affected nodules. As aforementioned, more cells appear to be phagocytized. In summary, the sections show suppurative granulomatous inflammation.

Group III: Scarification, 16th day postinjection.

A. Capsule and periglandular tissue

1. The capsule and the periglandular tissue are less involved than in the previous section. The vascularity is greatly reduced almost to normal. The granulation tissue of the capsule is maturing and there are only a few of the neutrophiles and macrophages remaining in the area. An occasional lymphocyte is present.

B. Lymph node

1. The inflammation is much more extensive than was observed in the 10th day section, involving the whole node. The germinal centers which were seen

in the previous section to be coalescing, are extensively coalesced into large areas of the gland. The central necrotic core is much more extensive, and the granulomatous response much less. In the center of the inflammatory nodule is an almost cell-free area of caseation necrosis, consisting of a pink granular amorphous debris. Examination of the periphery reveals a scattering of nuclear debris which is intermingled with the amorphous material of necrosis. The cellular debris builds up more and more towards the periphery until viable epitheloid cells and heterophiles blend in with the debris. In addition, a few lymphocytes and plasma cells are present. The outer area of epitheloid cells and heterophiles is much less extensive than in the three- and ten-day sections. Surrounding each of the inflammatory nodules are fibroblasts which are lining up, and some collagen formation as an initial walling off of the suppurating granulomatous foci. These strands are being laid down parallel to the surface of the abscess. Bacterial cells are found more numerous in the necrotic cores. They are found in their short-chain form. Towards the periphery of the nodules, smaller numbers of organisms are seen only as pairs and singles. A few are phagocytized near the area of the epitheloid cells. The section shows, in summary, beginning pus formation and more complete walling off.

Group III: Scarification, 30th day postinjection.

A. Capsule and periglandular tissue

1. The capsule is composed of well-vascularized, dense connective tissue with focal areas of accumulations of plasma cells, lymphocytes, and a few reticulo-endothelial cells. The capsule is definitely thicker than normal. Bands of connective tissue are extending into the lymph node from the capsule and trabeculae (Plate III).

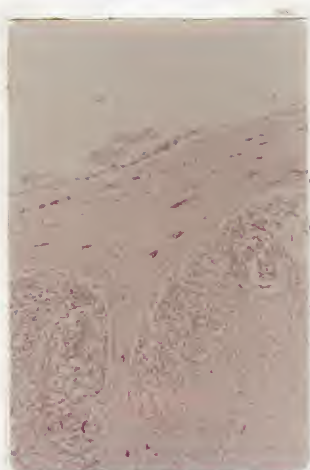
B. Lymph node

1. Few lymphocytes are present in the node, and they are replaced by fibroblasts, plasma cells, heterophiles, epitheloid cells, connective tissue, and cellular debris. Many plasma cells are scattered throughout the node. The reaction is similar to the previous section except that much of the debris has been liquefied and removed, leaving a clear area. Fibrosis is more extensive in the lymph node as evidenced by the strands of connective tissue running through the node, especially surrounding the necrotic areas. Bacteria are numerous only in the necrotic areas, where they are found unphagocytized. There are a few macrophages around the periphery of the node which contain ingested bacterial cells. Extensive suppuration and fibrosis would summarize the section.

EXPLANATION OF PLATE III

Capsular area of a cervical lymph node of an infected guinea pig (group III, scarification) 30-days postinjection, showing the thickened capsule composed of dense connective tissue. 125X.

PLATE III



Group I, subcutaneous injection, 3rd day postinjection. Changes seen in this section are similar to those observed in group III, 3rd day.

Group II, subcutaneous injection, 10th day postinjection. The capsule is thicker and shows more dense connective tissue than seen in group III, 10th day. There are a few fibroblasts and a few connective tissue fibers beginning to form around the margins of the nodules. Bacteria are seen in somewhat fewer numbers than in group III. Other changes resembled those seen in group III.

Group I, subcutaneous injection, 16th day postinjection. Not examined.

Group I, subcutaneous injection, 30th day postinjection. The microscopic lesions are similar to those of group III except the capsule is not as thick and there is less connective tissue lining the node.

Group II, intradermal injection, 3rd day postinjection. The lymph node is essentially normal. The reaction is still confined to the skin.

Group II, intradermal injection, 10th day postinjection. The reaction is very similar to group III except there seems to be more of the cortical area involved.

Group II, intradermal injection, 16th day postinjection. This section was identical to group III except the capsule and periglandular tissues are still undergoing exudative changes. A large number of heterophiles are still present along with macrophages, young fibroblasts, and lymphocytes. The vascularity

is still prominent.

Group II, intradermal injection, 30th day postinjection. This section was similar in many respects to group III except there appears to be more fibrosis of the gland. Several areas of the cortex in which there are inflammatory nodules, are surrounded with many connective tissue fibers.

The inflammation as seen microscopically, resembles proliferative inflammation as described by Smith and Jones (1961). The normal lymphocytes are rapidly replaced by proliferating epitheloid cells or reticulo-endothelial cells, which might also be called mononuclear macrophages. Early in the infection process this could well be called an infectious granuloma. The capsule uniformly undergoes proliferation and thickens. The organism although effectively walled off, apparently produces enough necrotoxins to destroy all of the cellular material of the node except the fibrous elements, and a large pus-filled abscess is created.

Feeding Trials

Nodules appeared in all animals of all infected groups in three days. The animals in groups E, F, and G did not develop any lesions. Group G had a severe death loss beginning on the tenth day following scarification. A gram negative rod and a staphylococcus were isolated from the pneumonia which was present in all of the dying guinea pigs. No organisms resembling the hemolytic mucoid streptococcus used in the experiment were found.

The losses stopped just as quickly as they had begun with only two animals remaining. One animal in group E died during the experiment due to injuries suffered by falling from the top cage where it was located.

Necropsies of the infected groups revealed enlarged lymph nodes only. No other gross pathology was observed. Animals showing enlarged nodules all contained the hemolytic mucoid streptococci. Organisms were not isolated from the other organs. Guinea pigs which did not show enlarged lymph nodes were bacteriologically negative for the hemolytic streptococcus.

The following table shows the results of the necropsies, and the relative lymph node size.

Table 1. Feeding trial results.

Group number	Number showing nodules	Per cent infected	Number nodules per G.P.	Sq. cm. of abscess
A	8 of 9[1]	88.8	2.0	3.215
B	7 of 10	70	1.28	1.028
C	2 of 9[2]	22	1.5	2.82
D	6 of 9[3]	66	2.0	2.11

[1] One animal died in group A 24 days postinjection. The death occurred during a hot weekend, and when the animal was found it was very decomposed. No nodules were seen, and postmortem decomposition precluded a bacterial examination.

[2] One animal died three days postinjection. The hemolytic streptococcus was isolated from the lymphnode, heart blood, liver, spleen, and kidney.

[3] One animal died seven days postinjection, and on necropsy an enlarged lymph node was found and a severe fibrinous pneumonia. The inoculated organism was isolated from the lymph node, lung, and liver.

From the foregoing table it is evident that there was an appreciable reduction in the numbers of infected animals in group C which is the group which received the combination of ethylenediamine dihydroiodide and sulfanilamide. It is interesting to note that the percentage of affected animals in the control group is less than either group A or B. There are fewer nodules per animal in group C than in the control group or group A, but its relative significance is very slight. The total surface area of abscesses produced in the inoculated groups does not correlate with the percentage affected, but again its relative significance is slight because the experiment is designed primarily to find a treatment to completely eliminate the infection from an animal.

It appears that the combination of the two drugs is more satisfactory in controlling and eliminating the infection in lieu of using either one by itself. Karel et al. (1941) reported that sulfanilamide was an effective treatment for this disease; however, they were using the criteria of survival time as an indication of effectiveness. This certainly does not hold true under field conditions, as we see that group D, the infected non-treated control, had only a slight death loss yet 66 per cent of the animals remained infected. The goal should be to control the disease and eliminate the organism completely from animals in a colony.

Hematology

The average blood cell counts of the 21 guinea pigs from all groups to be used as normals are as follows:

Red blood cells	5,478,000
White blood cells	9,500

The average white blood cell differential count was as follows:

	Per cent
Lymphocytes	48
Neutrophiles	42
Immature neutrophiles	0.5
Eosinophiles	0.2
Basophiles	0.04
Monocytes	9

These average counts approximate very closely those of Bilbey and Nocol (1955), except the differential count differed in some respects. There were 16 per cent less lymphocytes and 10 per cent more neutrophiles than those of the aforementioned authors. They found only 3.1 per cent monocytes where 9 per cent were found in this experiment. The eosinophiles, basophiles, and immature neutrophiles showed only insignificant differences. Two days following the scarification and inoculation of groups A, B, C, and D, the average of the total white counts of the four groups climbed above the preinjection normal of 9,500 W.B.C.s and stayed consistently above this during the whole 30-day period. The peak of the average infected white blood cell counts was 16,000

which occurred on the 11th day postinjection. This was true except for a period of six days during the last eight days of the experiment when the external environmental temperature rose very high for a period of about 10 days. The peak at this time rose to 18,000 W.B.C.s. It is felt that the high external temperature might well have affected this rise in count.

The average white blood cell counts of the non-infected groups E, F, and G also rose above the preinoculation level of 9,500 cells and closely paralleled the average white blood cell curve of the infected groups, except on the 11th day when the count fell to 9,000 W.B.C.s.

The average percentage of lymphocytes of the infected groups was consistently below the preinjection level of 48 per cent--ranging from a low of 28 per cent to a high of 47 per cent. The period of the 8th through the 11th day was the time of the lowest percentage of lymphocytes. The average percentage of lymphocytes from the non-infected groups remained more nearly in the normal range.

The average percentage of neutrophiles of the infected groups was consistently above the level of 42 per cent--ranging from a high of 64 per cent to a low of 36 per cent. The period of the 8th through the 11th day showed the highest consecutive percentages. The average percentage of neutrophiles of the non-infected groups remained closer to the normal average. The first five days showed a below normal percentage of neutrophiles with a slight increase in the percentage from that time on.

The average percentage of monocytes of both the non-infected and the infected groups remained fairly close to the normal range.

The average percentage differences of eosinophiles, basophiles, and immature neutrophiles were insignificant.

The daily white blood cell counts, red blood cell counts, and differential cell counts were tabulated from each group in the infected groups. There were no significant changes except for minor day-to-day variations from one group to another. This was also true of the non-infected groups.

The only significant finding of the white blood cell study was the fact that the count remained elevated above normal throughout the entire 30 days of the experiment. This was true of all groups. The infected groups did, however, remain higher in total white cell counts than did the non-infected groups. The neutrophiles remained higher in percentage than the average preinoculation normals, and the lymphocytes remained lower in percentage than the normals in the infected groups. This was not due to a lymphopenia, but due to a high neutrophilia. It was interesting to note that the neutrophiles and lymphocytes remained in a nearly normal percentage range in the non-infected groups even though a leucocytosis was evident.

The red blood cell count varied slightly from the average normal of 5.4 million. The infected groups A, B, C, and D peaked at 6.3 million R.B.C.s 11 days post inoculation and hit a low of 4.8 million R.B.Cs at 18 days post inoculation. The average R.B.C. counts of the non-infected groups peaked at 6.1 million on the 1st, 8th, and 9th days. The low average was reached on the

18th day at 5.1 million. There were no significant changes as evidenced by these data. When broken down into individual groups, the results of each group paralleled very closely to that shown in the average of the infected groups and the average of the non-infected groups.

The results of the red blood cell counts reveal no significant change during the disease process. Hardenbergh (1926) mentioned that in the disease process the blood picture was variable and showed no significant change. This was generally true except for a rise in total white cell counts in the infected groups and the increased percentage of neutrophiles present. The red blood cells were not altered significantly. The results of the red cell counts also revealed in the non-infected groups that the drugs used in the experiment had no effect on the total red blood cells.

Bacteriology

Microscopic examination of a Gram stained smear of the organism from a blood agar plate revealed a Gram-positive coccus in chain lengths of three to five organisms. Singles and pairs were also observed. In broth cultures, the organisms were found in chains of 5, 10, and up to 20 organisms in length. Capsules were not observed. The following observations were made from the agar plates with 5 per cent blood of the various species:

24 hours

Ox blood: Colonies were 1.5 mm in diameter, round, smooth, amorphous, entire, transparent, and glistening.

They were sticky and had a tendency to run together. Beta hemolysis was present and clear cut (Plate IV).

Guinea pig blood: Same as above.

Human blood: Colonies were about 1 mm in diameter, otherwise same as above.

Rabbit blood: A more narrow zone of beta-hemolysis was seen. The colonies have more of a tendency to be elliptical in shape.

Horse blood: Colonies were 1 mm in diameter and elliptical.

48 hours

Ox blood: The colonies were 2 mm in diameter, spreading with an opaque center, very moist and sticky, more irregular in shape, and a very clear beta hemolysis.

Guinea pig blood: The hemolysis was not clear. The colonies were 2.5 mm in diameter and more elliptical.

Human blood: The colonies were 1.5 to 2 mm in diameter, showing clear beta hemolysis. The colonies formed more opaque centers.

Rabbit blood: Colonies were 0.75 mm in diameter and more elliptical in shape.

Horse blood: The colonies showed very clear beta hemolysis, 2 mm wide, elliptical in shape, moist and sticky with some coalescing.

All four isolates used, gave identical results.

EXPLANATION OF PLATE IV

An ox blood agar plate with a 24-hour culture of the mucoid hemolytic streptococcus, the organisms of etiology. The prominent large wet colony and the distinct beta-hemolysis are quite evident.

PLATE IV

Table 2 summarizes the results of the biochemical and serological reactions of the four isolated organisms.

Table 2. Biochemical and serological results.

Test utilized	Results
1. Lancefield serological typing	Type C
2. Fibrinolytic test	Negative
3. Catalase test	"
4. 10 per cent bile	No growth
5. 40 per cent bile	" "
6. Bile solubility	Not soluble
7. 2 per cent NaCl broth	Growth
8. 6 per cent NaCl broth	No growth
9. Nitrate reduction	No reduction
10. Gelatin liquefaction	No liquefaction
11. 1 per cent methylene blue milk	No growth
12. Litmus milk	Acid in 24 hours, no coagulation or digestion
13. 5 per cent sucrose	Growth, but not viscid
14. pH 9.6 broth	No growth
15. Sodium hippurate	Not hydrolyzed
16. Starch agar	Hydrolyzed
17. Esculin	Split
18. Lactose	Fermented
19. Sucrose	"
20. Sorbitol	"
21. Salacin	"

Table 2 (concl.).

Test utilized	Results
22. Galactose	Fermented
23. Dextrose	"
24. Maltose	"
25. Trehalose	Not fermented
26. Inulin	" "
27. Raffinose	" "
28. Glycerol	" "

The isolates grew well at 37° C. They failed to grow at 10° and 45° C. After 30 minutes at 60° C. they were killed. It was found early in pilot studies that a sugar-free medium containing beef extract had to be used because of the fastidious nature of the organism.

All four mice, three rats, and the rabbit died in 24 hours post inoculation. The one remaining rat died within 36 hours of the time of injection. The typical hemolytic streptococcus was isolated from the heart blood of all animals.

The results of the bacteriological and serological tests, and animal inoculations reveal the organism to resemble _Streptococcus zooepidemicus_ as described by Breed et al. (1957).

SUMMARY AND CONCLUSIONS

Gross pathology was confined in all cases to the lymph nodes of the head and neck except one. The one exception revealed two

small abscesses of the spleen. Typical abscessation of one or more lymph nodes was observed. The typical hemolytic mucoid streptococcus was isolated in all instances from only the abscesses. The disease process revealed in the gross findings, first an edema of the gland and periglandular tissue with necrotic foci beginning to be observed on the 10th day postinjection, with abscess formation beginning on the 16th day and continuing through the 30th day.

The histopathological examination of the regional lymph nodes revealed a hyperemia and edema of the capsule during the first three days of infection. The node itself, during this period, was undergoing a marked lymphoid depletion, with the germinal centers primarily involved. These cells were replaced by large mononuclear cells and granulocytes. Edema and exudative changes were present. Many streptococci were present.

On the 10th day of infection, the capsule was beginning to proliferate profusely. The node germinal centers appeared as poorly circumscribed coalescing inflammatory nodules blending into one another. They characteristically consisted of a central dense cellular core and an outer exudative proliferative area. The section revealed a suppurative granulamatous inflammation.

The 16th-day postinjection node revealed connective tissue from the capsule extending down into the cortex of the gland. The capsular connective tissue was maturing. The lymph node revealed inflammation involving almost the whole gland. Caseation necrosis was evident. The section, in summary, shows early exudate formation and fibrosis.

The last of the series, the 30th-day postinjection, revealed a capsule of dense thick connective tissue with more mature bands extending down into the gland. The gland appears almost completely as a large abscess.

From the overall picture of the histopathology, one would surmise the infection to be a proliferative or granulamatous inflammation with later abscess formation.

The feeding trials revealed that the group fed sulfanilamide and ethylenediamine dihydroiodide had the lowest incidence of abscess formation at the end of the 30-day feeding trial with a 22 per cent infection or two animals of nine affected. The group fed the sulfa had only a 70 per cent infection while the group fed the organic iodide had a 88.8 per cent infection. The infected non-medicated controls had a 66 per cent infection rate. The roughly estimated size of the abscesses did not correlate in total size as with the number infected in a group. The number of abscesses per guinea pig did not correlate with the number infected within the groups in the feeding trials.

The hematological results were largely inconclusive with a leucocytosis being produced in both the infected and the non-infected groups. The leucocytosis was significantly higher in the infected groups. The percentage of neutrophiles increased in the infected groups, and the percentage of lymphocytes decreased somewhat. The differences of the total white blood cell and red blood cell counts of individual groups, as compared to each other, were insignificant in both the infected and the

non-infected groups. The hematological findings did reveal no adverse effects on the hemopoetic activity of the guinea pig from feeding of the sulfa or organic iodide. The author feels that further work could be accomplished by using the two drug preparations in different levels to find a dosage rate, and schedule which might result in a near 100 per cent effectiveness. This information might be incorporated into a commercial feed to control this disease in a laboratory colony.

The etiology of the disease studied was Streptococcus zooepidemicus as indicated by the Lancefield grouping, tolerance tests, biochemical reactions, morphology of the organism on the slide and on the colony, and animal pathogenicity.

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STUDIES ON THE ETIOLOGY, PATHOLOGY, AND CONTROL
OF GUINEA PIG LYMPHADENITIS

by

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Lymphadenitis of guinea pigs has long been a problem in animal colonies maintained for research. This disease may enter a colony in a rather insidious way--suddenly appear and disrupt experimentation in progress. There is a greater emphasis being placed on disease-free animals for more exacting research, and for this reason more information on this disease and its control should be obtained.

Three phases of the disease were studied, i.e., etiology, pathology, and control. To study the pathology, three groups of guinea pigs were infected by three different routes: subcutaneously, intradermally, and by scarification. They were necropsied at 3-, 10-, 16-, and 30-days post inoculation. Gross pathological lesions were noted, and grossly enlarged lymph nodes if present, regional lymph nodes if not enlarged, and other organs were cultured. Sections were also obtained for histopathological examination.

Control and further studies on the pathological process by the use of hematology, were studied by infecting four groups of guinea pigs: A, B, C, and D with the hemolytic streptococci by scarification. When the animals revealed evidence of the disease, they were fed medicated feed. Group A was fed ethylenediamine dihydroiodide; group B was fed sulfanilamide; and group C was fed a combination of A and B. Group D served as an infected non-treated control. Three more groups of animals: E. F, and G served as non-infected controls. Group E received no medication; group F was fed sulfanilamide; and group G received ethylenediamine dihydroiodide. Blood samples were obtained from one guinea pig

from each group daily for 15 days and every other day for the remainder of the 30-day period. Total white blood cell counts, differential counts, and total red blood cell counts were determined.

The organism involved was isolated from four naturally occurring cases of lymphadenitis and examined by the use of biochemical tests, morphological characteristics, and animal inoculations.

The gross pathological findings revealed the presence of enlarged regional lymph nodes of the head and neck. The nodes early in the disease were firm and edematous. As the disease progressed, necrosis occurred and abscessation finally resulted.

Histopathology of the above nodules showed an early marked depletion of lymphocytes. They were replaced by reticulo-endothelial cells and heterophiles resembling a proliferative or granulomatous inflammation. Necrosis and pus formation became more evident, and capsule proliferation occurred. The final result was an abscess walled off by a dense connective tissue capsule.

The feeding trials resulted in group C having the least infection as evidenced by only 22 per cent of the animals having the typical abscesses. They were fed a combination of sulfanilamide and ethylenediamine dihydroiodide. Hematological findings were relatively inconclusive except for a marked leucocytosis and neutrophilia. The red blood cell counts were unaffected either by the infection or the drugs used.

The etiology of the disease, as indicated by serology, tolerance tests, biochemical reactions, morphology of the organism, and animal pathogenicity was Streptococcus zooepidemicus.