1958

An Organism Resembling Sphaerophorus necrophorus Isolated From a Beef Liver Abscess

James C. Canada

AN ORGANISM RESEMBLING *STREPTOCOCCUS NECROPHORUS* ISOLATED FROM A BEEF LIVER ABSCESS

By

James C. Canada

A thesis submitted
in partial fulfillment of the requirements
for the degree Master of Science at South
Dakota State College of Agriculture
and Mechanic Arts

July 1958

AN ORGANISM RESEMBLING *SPHAEROPHORUS NECROPHORUS*

ISOLATED FROM A BEEF LIVER ABSCESS

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

ACKNOWLEDGMENT

The author wishes to express sincere appreciation to his wife, Dawn, for her encouragement and assistance throughout the preparation and writing of this thesis.

Appreciation is also expressed to Dr. E. C. Berry and Dr. H. E. Calkins for their technical advice and assistance.

Joe

TABLE OF CONTENTS

LIST OF PLATES AND MEDIA COMPOSITION

INTRODUCTION

Condemned beef livers cause a loss of several million dollars each year. This loss is felt not only by the meat packer, but by the animal husbandryman as well as the consumer. Percentagewise, the United States annually condemns in federally inspected meat packing plants between two and 20 percent of all beef livers. These beef livers are principally of three pathological types. These types include liver abscesses, telangiectasis, and the "sawdust liver. Throughout the United States the liver abscess is the most common. It is characterized by white abscesses varying from microscopic size to a diameter of four inches. These abscesses are very odorous, cheesy in appearance, and often under pressure. The second type of nonprocessable liver is due to telangiectasis. Jensen, Frey, Cross, and Connell (24) write, "Telangiectasis is a condition of liver characterized by a single or multiple dark red foci throughout the parenchyma, resulting from vascular congestion and hemorrhage in the foci." These investigators also write of the third liver condition. "'Sawdust' is a meat inspectors' term and refers to a condition of liver characterized by single or multiple gray colored foci, each one to two millimeters in diameter."

Little is known of the three liver conditions in regard to their causative agents. By microscopic examination usually one finds gram-negative, granular filaments approximately 100 microns in length present in the exudate from the abscessed livers. Microorganisms of significance are seldom found in the telangiectasis and "sawdust" livers.

This investigation concerns an organisms isolated from a beef liver abscess.

LITERATURE REVIEW

The microorganism that is usually considered to be the causative agent of beef liver abscesses is known by a number of names. Bergey (4) names the organism Sphaerophorus necrophorus (Flugge, 1886) Prevot, 1938. Bergey's Manual of Determinative Bacteriology also lists the following synonyms: Bacillus necrophorus Flugge, Bacillus diphtheriae vitulorum Flugge, Streptothrix cuniculi Rohmerl, Bacillus funduliformis Malle, Bacillus thetoides Rist, Actinomyces necrophorus Lehmann and Neumann, Cohnistreptothrix cuniculi Chalmers and Christopherson, Bacteroides funduliformis Bergey, Spherophorus funduliformis Prevot, and Necrobacterium necrophorus Jonsen and Thjötta.

In addition, Wilson and Miles (44) offer the following synonyms: Schmerl's bacillus, Bang's necrosis bacillus, and Funiformis necrophorus. Dack and co-workers (8) add the synonyms, Bacterium necrophorus and Bacterium funduliforme. One can readily visualize the difficulty that arises in securing information from the literature, concerning the liver abscess organism, under such a taxonomical condition.

Morphologically speaking, Sphaerophorus necrophorus appears as either short or long rods and filaments with rounded ends. The filaments may reach a length of 100 microns (41). The filaments appear to have granules or septa. Wilson and Miles (44) claim that branching does not occur, while Bergey (4) writes that some authors deny this. The bacterium is easily stained with aniline dyes and is gram-negative.

While the investigators are not in complete disagreement regarding morphology, little harmony exists concerning the cultural character-

istics of Sphaerophorus necrophorus. Matsushima (30) writes that he and his co-workers were unable to cultivate this species in vitro, while Bergey (4), Wilson and Miles (44), and Smith (41) list a host of favorable media for the propagation of Sphaerophorus necrophorus. Orcutt (34) writes that the organism "....grows well on ordinary media plus a little serum." Schrivner (38) disagrees and claims that this isolate is very difficult to grow.

Dack and co-workers (7) found that the colonies of Sphaerophorus necrophorus when grown on blood agar were greyish, raised, and smooth. The colonies may grow to a diameter of 5 millimeters (41). Broth cultures of the organism are uniformly turbid with a slight, fine, dirty-white sediment (31). Newton (32) has successfully used brain medium for the propagation of the bacterium. West and co-workers (43) write that "....unknown substances, which occur in tryptone-glucose-cysteine medium but are not present in the synthetic media, are required by certain strains...."

Generally, it has been found that glucose, fructose, and maltose are fermented with acid and gas by Sphaerophorus necrophorus. Mannitol, sorbite, arabinose, salicin, trehalose, rhamnose, and xylose are not fermented (7). Bergey (4) and Dack and co-workers (7) write that sucrose is not fermented, while Wilson and Miles (44) claim that this characteristic is variable. Bergey (4) and Dack and co-workers (7) also write that glycerol is not fermented, while Smith (41) has reported fermentation of glycerol. Indole is formed from tryptophane (4, 7, 41, 44), hydrogen sulfide is formed (4, 41, 44), lead acetate is darkened (7), but nitrates

are not reduced to nitrites (4, 44). Smith (41) and Bergey (4) record that gelatin is not liquified, while Duck and co-workers (7) have found liquefaction in two of nine strains. Duck and co-workers (7) and Smith (41) write that no change occurs in litmus milk, while Wilson and Miles (44) have found a soft clot to be present, and Bergey (4) records that coagulation is present when the medium is inoculated with the organism. Reactions to both the methyl red and Vouges-Proskauer tests are negative (44).

Cultivation of the organism is difficult unless a fermentable carbohydrate is present, although at times serum or cysteine may be substituted (41). Grant (15) found that a medium containing only glucose, casitone, sodium chloride and L-cystine was nutritionally sufficient for growth of Sphaerophorus necrophorus. The organism is a strict anaerobe (38). Duck and co-workers (7) have grown the organism by exhausting the atmospheric pressure to four or five centimeters of mercury and then admitting carbon dioxide. Some strains are hemolytic to the erythrocytes of sheep, horses, cattle, rabbits, and humans (41). It has been noticed that a green zone surrounds colonies of Sphaerophorus necrophorus grown on blood agar when exposed aerobically (7, 41). Smith (41) writes that it is not uncommon for cultures to exhibit a lag phase of several days during routine transfers. This lag phase might be accounted for by the fact that hydrogen peroxide is produced when the organism is exposed to the air (17, 16, 18).

Beef livers that contain abscesses are condemned in federally inspected meat packing plants throughout the United States. In 1937, 10.9 percent of all beef livers in Colorado were condemned because of

abscesses (32). Smith (41) writes that between ten and 20 percent of all beef livers are condemned for human consumption due to abscesses. In a study of 56 abscessed livers, Madin (29) found that 89.2 percent contained Sphaerophorus necrophorus. In 1942, 5.25 percent of all cattle slaughtered in federally inspected meat packing plants in the United States had liver abscesses, according to Smith (40). He also writes that the Denver market condemned 13.3 percent, cities on the West Coast, between 10.7 and 11.7 percent. The Ft. Worth area is the lowest nationally with a 2.9 percent condemnation (40). Smith (40) estimated this loss to be two million dollars annually in the United States. According to the Meat Inspection Division of the United States Bureau of Animal Industry, in 1943 the annual loss resulting from condemned livers was $2,601,355.20 (14).

Sphaerophorus necrophorus has been known by its synonyms for many years. Dammann published work in 1876 describing his investigations of calf diphtheria (39). The organism itself, however, was first studied, in 1884, by Loeffler who had isolated it from a case of calf diphtheria (44). Loeffler inoculated mice subcutaneously with the diphtheric membrane and was able to produce necrotic lesions. He was also successful in obtaining a primary culture of the organism from the mice and in cultivating it in calf serum, but failed in attempts to subculture the bacterium. Shutz, in 1888, found the filamentous bacteria in the livers of cattle and succeeded in transferring the bacteria to rabbits and mice (5). One year later, in 1889, Theobald observed similar organisms in the intestinal lesions of hog cholera (5). Schmorl, in 1891, found similar organisms in an epizootic in which necrosis of the lower lip was

spread among laboratory rabbits (44). Schmorl named his isolate, Streptothrix cuniculi. While working with their isolate, Schmorl and an assistant developed lesions on their hands (39). This was the first time that Sphaerophorus necrophorus had been recorded from a human source. Veillon and Zuber, in 1897, worked with other pathogens of this genus (41). In 1898, Halle studied a bacillus, now thought to be Sphaerophorus necrophorus, associated with genital infections of man (20). Halle named the organism Bacillus funduliformis. Sphaerophorus necrophorus was first definitively isolated and cultured from a human source by Stemen and Shaw (39) in 1910. They isolated their bacterium from a lesion which had developed on the hand of a meat inspector. The meat inspector had previously scratched his hand on a sheep's tooth while examining an abscess on the lip of the animal.

Sphaerophorus necrophorus and related microorganisms attack many animals, including man, and produce a variety of pathological conditions. While this paper is mainly concerned with liver abscess infections of beef cattle, it is also interesting to note these other conditions. Buck and co-workers (7) write that the organism is a common inhabitant of the intestinal tract of man, monkeys, and baboons. The organisms find favorable conditions for growth in ulcers of the intestines. Buck (6) also writes that the organism is probably a normal inhabitant of the mucous membranes of man and animals. Kelly (25), through her investigations, writes that the bacterium was not found in the healthy oropharynx of monkeys, nor did it appear in artificially produced necrotic lesions of that region. Orcutt attributes to this organism the following pathological conditions found in animals (34): "....calf diphtheria, necrotic ulcers of

the intestine in hog cholera, foot-rot in sheep and cattle, grease heel or necrotic scratches of horses, lip and leg ulceration of sheep, gangrenous dermatitis of horses, metastatic necrosis of liver and lungs of cattle and swine, necrotic stomatitis of calves, lambs and pigs." Flint and Jensen (13) add that cattle suffer from arthritis of the coffin joint which is also due to Sphaerophorus necrophorus. Fitch (11) writes that this agent causes five principal diseases in swine: necrotic stomatitis, necrotic rhinitis, necrotic gastritis and enteritis, necrotic dermatitis, and necrotic pneumonia. An Australian investigator, Harris (19), has recorded observations of the organism in cases fatal to young lambs between seven and ten days old. Smith (41) lists the following animals known to be infected with Sphaerophorus necrophorus: horses, cattle, pigs, sheep, goats, chickens, rabbits, reindeer, kangaroos, monkeys, and antelope. Wilson and Miles (44) claim that the organism is not pathogenic for guinea pigs, dogs, cats, pigeons, and hens. On the other hand, the bacterium has been studied from lesions from the heads of chickens (10). Boyd (3) has isolated the organism from cases of "sore mouth" in tortoises and snakes. Rosen and co-workers (36), in writing of foot rot due to Sphaerophorus necrophorus, claim that it "....constitutes a serious threat to the continuous existence of deer on some of the ranges of California."

Smith (41) describes the beef liver abscesses as being from a few millimeters to several centimeters in diameter, yellow-white in color, consisting of central necrotic caseous masses surrounded by large numbers of chronic-type reactive cells with zones of polymorphonuclear leucocytes. The filamentous bacteria are found primarily in

the leucocyte zone within a capsular layer of connective tissue fiber. Jensen, Flint, and Griner (22) write that in the early stages of acute abscess formation the animals are sick and may die. In chronic stages of abscess formation and throughout the entire course of the disease, the animals are not clinically sick and, consequently, continue to eat and gain weight (22).

Pathological conditions have arisen in human cases due to Sphaerophorus necrophorus and related organisms. The organism is considered to be a normal inhabitant of the mouth and intestinal tract (44). The human strains of the bacterium are similar to the animal strains with the exception of lower pathogenicity to laboratory animals (44). Sphaerophorus necrophorus has been observed in the following pathological conditions of humans: localized superficial lesions, female genital tract, bacteremia, menengitis, empyema, lung abscesses, bronchiectasis, urinary tract infections, chronic otitis media, maxillary sinusitis, osteomyelitis, cervical sinuses, abdominal abscesses, and thyroiditis (1). Antibodies to the organism have been found in the blood of individuals suffering from chronic ulcerative colitis but not in the blood of healthy persons (41). Human cases of Sphaerophorus necrophorus infection ending fatally have been recorded (5, 28). Wilson and Miles (44) write that the mortality rate in cases of humans suffering from well-established infections of the organism is over fifty percent. Several suggestions have been proposed as to the possible route of infection of Sphaerophorus necrophorus. Newson (32) wrote that liver abscesses were believed to be caused by the feeding of beet by-products to cattle.

The organisms were absorbed through the digestive tract and carried to the liver through the portal circulation. Dack and co-workers (7) claim that the bacterium finds favorable conditions in the intestines. Jensen and co-workers (24, 23) believe, however, that the organisms enter the liver by the portal blood stream via a lesion of the rumen. They write, "In fattened beef cattle, rumenitis occurs commonly. The lesions probably lower efficiency of utilization of feed. Through foci of injury, bacteria, especially Sphaerophorus necrophorus, are able to penetrate the rumen wall, enter the portal blood, and be carried to the liver where secondary foci of infection may become established (23)." This theory seems entirely feasible as Robinson, Jasper, and Guilbert (35) have isolated Sphaerophorus necrophorus from the rumen in their experiments. Tunnicliff (42) has experimented with the organism as to its possible route of infection in regard to foot rot of cattle. He concludes that Sphaerophorus necrophorus is not a natural inhabitant of the soil and remains there only a short time. Frederick (14) reports of his experiments the same characteristic behavior of the organism. After investigating young lambs which had died of the Sphaerophorus necrophorus infection, Harris (19) writes, "It would seem that the lambs probably become infected by way of the umbillicus, probably during the first 24 hours after birth; that the infection localized in the liver, but that only after the lesions had developed during a period of several days was there sufficient disturbance of function to cause symptoms and ultimately death." It is interesting to note that Smith (41) found that in Denver liver abscesses are most common from September to

January with the peak of infections occurring in November. Possibly this fact may bear some relation to the route or routes of infection of Sphaerophorus necrophorus.

The agglutination test is not an aid to the diagnosis of an infection of Sphaerophorus necrosis (39). Titres of 1:800 are found in the blood of normal cattle, horses, sheep, and swine (44). Mack and co-workers (7) have found that strains of the organism are antigenically heterogenous.

Smith (41) claims that immunization of experimental animals was attempted but that it yielded an immunity of low order and that sporadically. Beveridge also was unable to demonstrate immunity (7). His investigations employed rabbits which had received two subcutaneous doses of formalinized culture. Jensen and co-workers (22) write, "Sheep were not protected by multiple intraperitoneal injections of sterile polyvalent culture filtrate of S. necrophorus against a challenge dose of viable S. necrophorus inoculated intraportally." Elder, Lee and Scrivner (9) report, however, that they succeeded in preparing an antiserum which would protect rabbits against experimental inoculations of the organism. Their work in immunizing calves has not been successful.

Sphaerophorus necrophorus has been reported to produce a soluble exotoxin (2, 31, 7) and a soluble endotoxin (2, 31, 7, 44). Merchant and Packer (31) found that the exotoxin produced an edema when inoculated intradermally into rabbits. The ability to produce lesions in animal tissue is largely due to the production of a necrotizing endotoxin found in the cells of the isolate (44).

In nature Sphaerophorus necrophorus is rarely, if ever, found in pure culture. This association with other microorganisms significantly increases the bacterium's virulence to the host (21). Beveridge (2) reports that Sphaerophorus necrophorus is much less susceptible to aerobic conditions when it is associated with either Micrococcus pyogens variety aureus or Escherichia coli. Schrivner and Lee (38) have found that virulence of the liver abscess isolate is increased when injected into rabbits if it is in a mixed bacterial flora. Smith (41) writes that other organisms increase in virulence when the endotoxin of Sphaerophorus necrophorus is added.

Information on the control and prevention of Sphaerophorus necrophorus infections is scanty. Fitch (11) claims that the proper disposal of manure would lower infections due to the organism. Ryff and Lee (37) write, "Marked vitamin deficiency increased the severity of the lesions observed, but conversely, feeding cod liver oil or ascorbic acid did not decrease the severity under that for control groups." McCullough (27) found that a severe scorbutus had to be present in guinea pigs before there was a drop in resistance to Sphaerophorus necrophorus. In 1919, Nelschek (33) wrote in regard to the treatment of Sphaerophorus necrophorus caused foot infections in horses and mules, "....each form must be treated with one object in view: expose the organism to the air. This is easily accomplished with the knife." The sulfonamide drugs have been found to be beneficial in the treatment of rabbits with artificially produced lesions due to Sphaerophorus necrophorus (44). Shaw (39) adds that potassium iodide should be given internally for the treatment of visceral lesions and antiseptics should be applied in

cutaneous cases. He also writes that there is a possibility that x-ray may be beneficial in the treatment of both visceral and cutaneous lesions. Flint (12), in recent experiments, has found that materially fewer liver abscesses occur with continuous feeding of 70 milligrams of Aureomycin per animal per day. His investigations involved 1,895 cattle over a period of two years; 714 animals served as controls and 1,181 animals received the Aureomycin.

PROCEDURES, RESULTS, AND DISCUSSION

Isolation of the microorganism from a beef liver abscess

On the 31st. of July, 1957, infected liver material was obtained from newly slaughtered cattle at Morrell's Meat Packing Plant of Sioux Falls, South Dakota. Specimens from three types of nonprocessable beef livers were obtained. These types included the liver abscess, telangiectasis, and the "sawdust" liver. Material from the liver abscesses was collected by several methods, including the severance of whole intact abscesses (approximately golf ball size), contact swabbing with the abscess exudate, and direct inoculation with the abscess exudate. Material from the telangiectasis and "sawdust" livers was collected by direct swabbing of the meat and by the severance of the meat in approximately one inch cubes. All material collected was then sealed, refrigerated, and transported to the Bacteriology Laboratory of South Dakota State College of Mechanical and Agricultural Arts at Brookings, South Dakota.

After arriving at the laboratory the material was inoculated immediately into various culture media. These media included fluid thioglycollate, liver broth, nutrient broth, beef blood agar, rabbit blood agar, beef blood broth, rabbit blood broth, beef serum broth, and rabbit serum broth. The cultures were incubated both aerobically and anaerobically at 37 degrees Centigrade. Anaerobiosis was maintained by the use of a Brewer's Jar. Approximately two cubic centimeters of the abscess exudate were inoculated into a large rabbit subcutaneously.

After 48 hours of incubation, the *in vitro* cultures were examined for growth. Material from the liver abscess exudate supported growth of gram-negative, granular filaments, approximately 100 microns in length when grown in fluid thioglycollate and liver broth. The other media used did not support growth of significant microorganisms. At no time were microorganisms of any significance isolated from *in vitro* cultures inoculated with material from the "sawdust" and telangiectasis liver conditions. Attempts were made to subculture the microorganisms isolated from the liver abscess by *in vitro* passages. These attempts proved unsuccessful.

Blood was drawn at 24 hour intervals from the marginal ear vein of the rabbit inoculated from the liver abscess. On the third day of incubation and following, gram-negative granular filaments, approximately 100 microns in length, were cultured from the rabbit's blood stream in anaerobic fluid thioglycollate and liver broth. Again, further attempts to subculture the organisms proved unsuccessful. Emaciation of the rabbit appeared after the fifth day of incubation. Loss of appetite was noticed on the following day. On the seventh day of incubation the rabbit was autopsied. A large lesion containing a white, very odorous exudate was found at the site of the injection. Microscopic examination of this lesion revealed organisms similar to those cultured from the blood stream.

The entire procedure was initiated again in mid-August. The results were repeated with the same subculturing difficulties. Early in September, *in vitro* subculturing was again attempted. The original culture was a tube of liver broth inoculated with blood from a rabbit

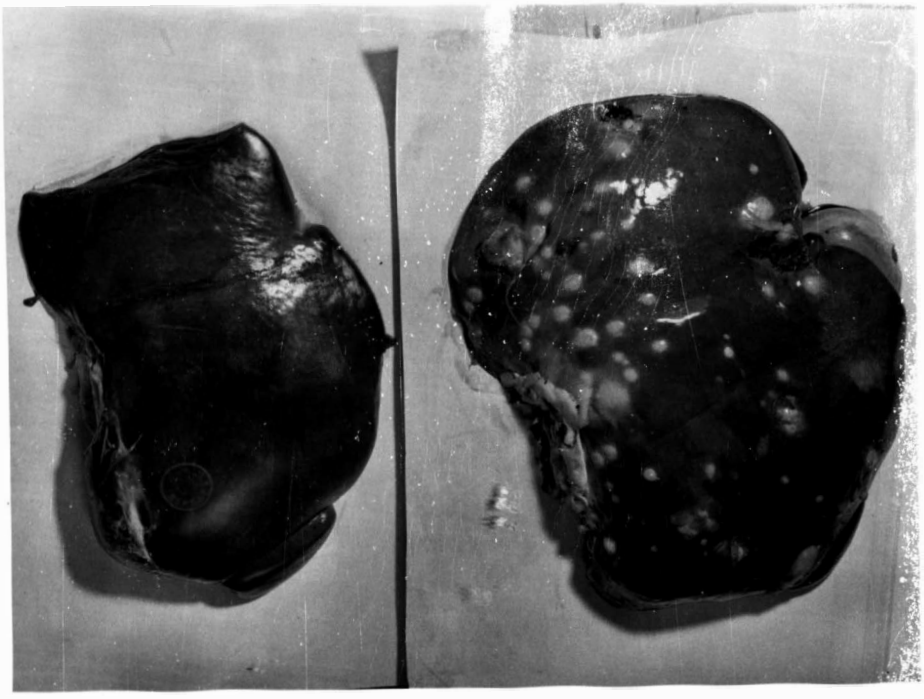

Plate I. Normal beef liver (left) compared with abscessed beef liver (right). (Courtesy of Charles Pfizer and Company, Terre Haute, Indiana).

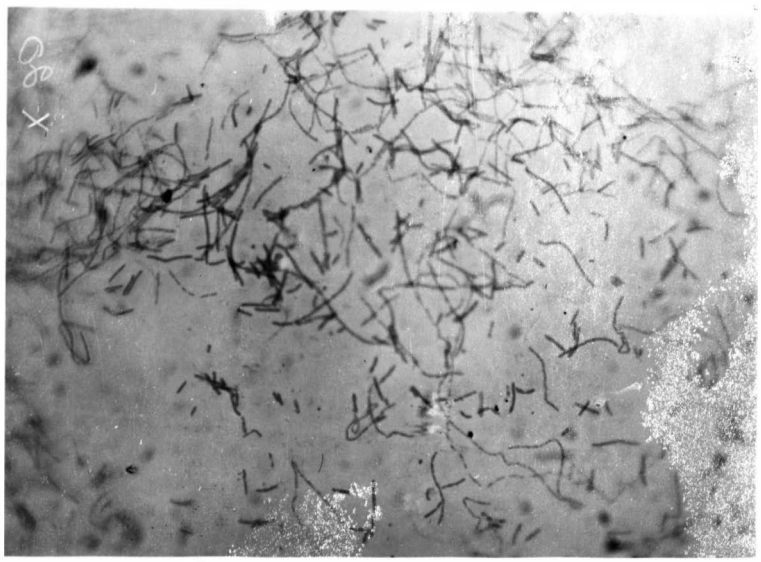

Plate II. The isolate magnified approximately 1000 times. Stained by Gram's method.

which was infected with the liver abscess organisms. The subculturing this time proved successful. Gram-negative granular filaments approximately 100 microns in length were cultivated. Subculturing from this culture was also successful.

It was feared, at this point, that with the newly acquired adaptation of cultivation in vitro, virulence may have been lost. This, however, was not true. A large rabbit was subcutaneously inoculated with a subculture of the organism. After five days of incubation the rabbit was autopsied. Gram-negative granular filaments, approximately 100 microns in length, were present both in the blood stream and in a lesion at the site of injection. These organisms had not lost their virulence through their adaptation to subculturing in vitro. Pure cultures of the organism were obtained by selecting colonies from fluid thioglycollate to which 0.2 percent agar had been added. Microorganisms originating from this strain were used in the following experiments.

Animal inoculations

Experiments were initiated to study the pathogenicity of the isolate in regard to lower animals. It had been found that laboratory rabbits succumbed to the infection between the fifth and seventh day of incubation. A large, very odorous, white, cheesy lesion was always present at the site of the subcutaneous injection. The causative organism could be cultured from either the blood stream or the lesion.

Chick embryos were inoculated with cultures of the isolate grown in fluid thioglycollate. Quantities of 0.1 or 0.2 cubic centimeter of the cultures were inoculated into the chorio-allantoic fluid of the embryos. Sterile fluid thioglycollate in the same quantities was also

inoculated by the same route in other embryos. The chick embryos which were inoculated with the culture died after 24 hours of incubation. The chick embryos that were inoculated with sterile fluid thioglycollate survived. Attempts to recover the organisms from the infected chick embryos proved unsuccessful.

Laboratory mice were injected both suboutaneously and intraperitoneally with cultures of the organism grown in fluid thioglycollate. Mice were also injected with sterile fluid thioglycollate by both routes. All mice receiving the sterile fluid thioglycollate survived. The mice that were injected intraperitoneally with the cultures died after 24 hours of incubation. These animals were autopsied, but nothing of significance was noticed. The mice which were injected subcutaneously survived without symptoms.

Laboratory rats were injected with the isolate grown in fluid thioglycollate both intraperitoneally and intratesticularly. The latter site of inoculation was an attempt to demonstrate a localized, but non-fatal, infection. Again, controls using sterile fluid thioglycollate showed no symptoms. The rats which were injected intraperitoneally received 0.2 cubic centimeter of the culture. These animals died within three days. Nothing of significance was observed upon autopsy. The animals which were injected intratesticularly were anesthetized and then inoculated in one testicle with 0.1 cubic centimeter of the culture. These animals showed no symptoms after five days of incubation. Upon autopsy nothing of significance was noted. The organism was not recovered in an *in vitro* inoculation into fluid thioglycollate.

Antibiotics

A preliminary experiment with the isolate in regard to antibiotics was initiated. The antibiotics used were penicillin, Aureomycin, bacitracin, neomycin, and tyrothrycin. A loop of the culture was inoculated into tubes containing 20 cubic centimeters of fluid thioglycollate plus three filter pad discs containing five milligrams percent each of the appropriate antibiotic. After 48 hours of incubation, growth was present in the tubes containing the neomycin and tyrothrycin. Growth was not observed in the tubes containing the penicillin, Aureomycin, and bacitracin.

Methods of preservation

During the experiments it was necessary to transfer the isolate under *in vitro* conditions every four days. There was considerable risk in transfer as contaminants might be introduced or a mutational change might occur. Naturally, it would be advantageous to preserve a stock culture. The first attempt of preservation employed sterile soft glass tubes to which 10 cubic centimeters of a culture of the isolate grown in fluid thioglycollate were added. The mouths of these glass tubes were heated to softness and then sealed under vacuum. Cultivation of the preserved organisms after several weeks proved unsuccessful.

Cultures of the isolate in both fluid thioglycollate and liver broth were frozen at a temperature of approximately minus 60 degrees Centigrade. Upon thawing, after several days of storage at this temperature, the cultures were found to be nonviable.

Finally, cultures of the organism were lyophilized in an attempt

to preserve them. One cubic centimeter of a heavy suspension of the isolate was added to an equal volume of sterile rabbit blood which had been defibrenated. Two drops of this mixture then were added to each of several sterile Durham tubes containing sterile ground glass. Then the Durham tubes were plugged with cotton and sealed inside soft glass tubes under vacuum. After several weeks of lyophilization, cultivation from these cultures was found to be unsuccessful.

Toxin

Experiments to determine the presence of a toxin were run according to the procedure of Smith (41). It had been observed that intraperitoneal injections of whole cultures were fatal to mice. It was suspected that either an exotoxin or an endotoxin or possibly both were present.

The exotoxin was prepared in the following manner. Five cubic centimeters of a fluid thioglycollate suspension of the organism were precipitated by the use of a centrifuge; 0.2 of a cubic centimeter of the supernatant were injected intraperitoneally into each of several mice. After three hours of incubation the mice appeared to be suffering from the effects of a virulent exotoxin. However, these mice survived this crucial period and fully recovered.

An endotoxin was prepared by resuspending, in sterile fluid thioglycollate, the cells which had been precipitated in the exotoxin experiment. The cells were once again precipitated by the use of a centrifuge and resuspended in sterile fluid thioglycollate. The next step was to precipitate the cells, but this time to resuspend the cells in sterile distilled water. The distilled water was used to lyse the cells and liberate any endotoxin which might be present. This suspen-

mice was then precipitated; 0.2 of a cubic centimeter of the supernatant were injected intraperitoneally into each of several mice. Again, as in the exotoxin experiment, the mice appeared to be affected by the injection after three hours. These mice survived this initial shock, only to die between the fifth and sixth days of incubation.

Nutritional studies

The final set of experiments was organized to determine the nutritional requirements of the liver abscess isolate. It had been found that the organism could be successfully propagated in either liver broth or fluid thioglycollate. (The composition of these media may be seen on page 26). Liver broth is difficult to prepare and of varying chemical composition. The beef liver should be soaked in one liter of tap water under refrigeration for approximately 12 hours. Then the fat is skimmed off the surface. Next the material is heated in the autoclave for ten minutes under 15 pounds of pressure per square inch. The meat is removed from the broth by straining the mixture through cheese cloth. This meat is saved for later use. Neopeptone and dipotassium phosphate are added to the broth which is then heated to 100 degrees Centigrade. Upon cooling, sufficient tap water is added to the mixture to raise its volume to one liter. The pH is adjusted to eight and the broth is strained through paper. Then culture tubes are filled with one-half inch of the previously strained meat and enough broth is added to raise the level to three inches. The medium is sterilized in an autoclave at 15 pounds of pressure per square inch for 15 minutes. Immediately before inoculation, tubes of the medium should be heated in a boiling water bath for ten minutes to remove the oxygen, then

quickly cooled. After inoculation the culture is sealed with paraffin

Since the liver broth has not been chemically defined, fluid thioglycollate was used as a basal medium. Thioglycollic acid is present to resist an oxygen uptake and resazurin serves as an aerobic indicator. Both of these constituents were eliminated from the nutritional basal medium.

The first medium prepared, "X", was made in an attempt to duplicate the results obtained with fluid thioglycollate. Neither casitone, a proteolytic digest of casein, nor L-cystine were available, so beef peptone, a proteolytic digest of beef, and L-cysteine hydrochloride was substituted. After inoculation and incubation no growth was found to be present in the "X" medium. The ingredients, for which substitutes had been used, were acquired and added in the preparation of "Y" medium. This medium was essentially fluid thioglycollate medium minus thioglycollic acid and resazurin. Growth occurred after inoculation and a suitable incubation period.

The next medium prepared, "ZZ", contained the basal medium minus yeast extract. Yeast extract is a good supply of vitamins and nutrients but is difficult to define chemically. Growth was observed in the "ZZ" medium, however. In "YY" L-cysteine was substituted for the amino acid, L-cystine. Growth was present after inoculation and incubation.

Beef peptone was substituted for the casitone in "Z" medium. This medium, containing the beef peptone, did not support growth of the isolate, however. "C" medium was composed of the basal medium ingredients but contained neither of the amino acids, L-cystine, or L-cysteine.

This medium did not support growth of the isolate. "D" medium contained the amino acid, L-cystine, but lacked the sugar, dextrose. No growth of the isolate was found to be present in this medium.

Media, "A" and "B", both contained substitutions for casitone. The former medium employed vitamin-free casein while the latter had ten percent casein hydrolysate. Growth of the culture was not present in either "A" or "B" medium after inoculation and incubation.

COMPOSITION OF MEDIA

Liver Broth

fresh ground beef liver	500 grams
tap water	1000 milliliters
neopeptone	10 grams
K_2HPO_4	1 gram

Thioglycollate Medium

casitone	15 grams
yeast extract	5 grams
dextrose	5 grams
sodium chloride	2.5 grams
L-cystine	0.5 gram
thioglycollic acid	0.3 milliliter
agar	0.75 gram
resazurin	0.001 gram
H_2O	1000 milliliters

"X" Medium

beef peptone	15 grams
yeast extract	5 grams
dextrose	5 grams
NaCl	2.5 grams
cysteine hydrochloride	0.5 gram
agar	0.75 gram
H_2O	1000 milliliters

"Y" Medium

casitone	3 grams
yeast extract	1 gram
dextrose	1 gram
NaCl	0.5 gram
L-cystine	0.1 gram
agar	0.15 gram
H_2O	200 milliliters

"ZZ" Medium

casitone	3 grams
dextrose	1 gram
NaCl	0.5 gram
L-cystine	0.1 gram
agar	0.15 gram
H_2O	200 milliliters

"YY" Medium

casitone	3 grams
yeast extract	1 gram
dextrose	1 gram
NaCl	0.5 gram
L-cysteine	0.1 gram
agar	0.15 gram
H_2O	200 milliliters

"Z" Medium

beef peptone	3 grams
yeast extract	1 gram
dextrose	1 gram
NaCl	0.5 gram
L-cystine	0.1 gram
agar	0.15 gram
H_2O	200 milliliters

"A" Medium

vitamin-free casein	3 grams
dextrose	1 gram
NaCl	0.5 gram
L-cystine	0.1 gram
agar	0.15 gram
H_2O	200 milliliters

"B" Medium

ten percent casein hydrolysate	30 milliliters
dextrose	1 gram
NaCl	0.5 gram
L-cystine	0.1 gram
agar	0.15 gram
H_2O	170 milliliters

"C" Medium

casitone	3 grams
dextrose	1 gram
NaCl	0.5 gram
agar	0.15 gram
H_2O	200 milliliters

"D" Medium

casitone	3 grams
NaCl	0.5 gram
agar	0.15 gram
L-cystine	0.1 gram
H_2O	200 milliliters

SUMMARY AND CONCLUSIONS

Future bacteriological research on the causative organism, or organisms, of beef liver abscesses is much in demand as indeed this paper illustrates. Much of the time that was spent by the author was consumed only by methods of propagating the isolate. Certainly, the research should consider more than one isolate. Classification and taxonomy of the isolated microorganisms should be more clearly defined as well as mode of transmission, physiology, and pathogenesis. Now there is evidence that antibiotics play a role in combating the infection. This therapeutic measure should be more fully investigated.

To summarize this paper we find:

1) A microorganism has been isolated from a beef liver abscess. This isolate resembles Sphaerophorus necrophorus (Flugge, 1886) Prevot, 1938 (4) morphologically but differs in physiological respects. It may be propagated *in vitro* without a loss of virulence to laboratory animals.

2) This author has unsuccessfully attempted to preserve the organism. The only method found to keep the isolate viable was frequent *in vitro* transfers or continued animal passage.

3) The isolate, when grown *in vitro*, is sensitive to penicillin, Aureomycin, and bacitracin.

4) It is suggested that an endotoxin may be present which is virulent to laboratory mice upon intraperitoneal injections. If an exotoxin is present, it is of low order in regard to virulence when injected intraperitoneally into laboratory mice.

5) The nutritive requirements of the isolate are satisfied by: casitone, glucose, sodium chloride, and either L-cystine or L-cysteine.

LITERATURE CITED

1. Beigelman, Paul M. and Lovell A. Rantz. Clinical significance of Bacteroides. Archives of Internal Medicine. 84:605-631. 1949.

2. Beveridge, W.I.B. A study of twelve strains of Bacillus necrophorus, with observations on the oxygen intolerance of the organism. Journal of Pathology and Bacteriology. 38:467-491. 1934.

3. Boyd, A.G. Observations on disease in captive wild animals, with special references to necrophorus infection in snakes and tortoises. The Cornell Veterinarian 19:33-39. 1929.

4. Breed, Robert S.; E.G.D. Murray and Nathan R. Smith. Bergey's Manual of Determinative Bacteriology. 7th Edition. The Williams and Wilkins Company, Baltimore, 1957.

5. Cunningham, John Sumter. Human infection with Actinomyces necrophorus. Archives of Pathology. 9:843-868. 1930.

6. Dack, G.M. Non-sporeforming anaerobic bacteria of medical importance. Bacteriological Reviews. 4:227-260. 1940.

7. Dack, G.M.; Lester R. Dragstedt and Theodore E. Heinz. Further studies on Bacterium necrophorum isolated from cases of chronic ulcerative colitis. Journal of Infectious Diseases. 60:335-355. 1937.

8. Dack, G.M.; L.R. Dragstedt, Robert Johnson and E.B. McCullough. Comparison of Bacterium necrophorum from ulcerative colitis in man with strains isolated from animals. Journal of Infectious Diseases. 62:169-180. 1938.

9. Elder, Cecil; A.M. Lee and L.E. Scrivner. Necrobacillosis of calves. Forty-second Annual Report of University of Wyoming Agricultural Experiment Station. No. 183. 1931.

10. Hamel, M.W. Necrophorus infection in chickens. Journal of American Veterinary Medical Association. 113:169. 1948.

11. Fitch, C.P. Necrobacillosis. The Cornell Veterinarian. 9:93-103. 1919.

12. Flint, J.C. Personal communication. May, 1958.

13. Flint, Jean C. and Rue Jensen. Pathology of the necrobacillosis of the bovine foot. American Journal of Veterinary Research. 12:5-13. 1951.

14. Frederick, L.D. The economic and nutritional importance of bovine hepatic disturbances. Journal of the American Veterinary Medical Association. 102:338-345. 1943.

15. Grant, Dale Walter. Nutritional studies on strains of Sphaerophorus necrophorus. Master's degree thesis. Colorado State University. 1953.

16. Hagan, William A. The reason for failure to obtain growth of an obligatory anaerobe (Actinomyces necrophorus) on plate cultures incubated in an anaerobic jar. Proceedings of the Society for Experimental Biology and Medicine. 21:568-570. 1923-24.

17. Hagan, William A. Formation of peroxide by Actinomyces necrophorus on exposure to air in relation to anaerobic plate cultures. Journal of Infectious Diseases. 35:390-400. 1924.

18. Hagan, William A. The formation of hydrogen peroxide by an obligatory anaerobe (Actinomyces necrophorus). The tolerance of an organism for peroxide. Proceedings of the Society for Experimental Biology and Medicine. 21:570-572. 1923-24.

19. Harris, A.H.A. Fusiformis necrophorus infection of the liver in young lambs. The Australian Veterinary Journal. 23:152-153. 1947.

20. Hawthorne, John C.; Luther Thompson and Donald C. Beaver. Gram negative bacilli of the genus Bacteroides. Journal of Bacteriology. 31:255-274. 1936.

21. Hite, Kileen E.; Marcia Locke and E. Closs Hesseltine. Synergism in experimental infections with non-sporulating anaerobic bacteria. Journal of Infectious Diseases. 84:1-9. 1949.

22. Jensen, Rue; Jean C. Flint and Lynn A. Grimer. Experimental hepatic necrobacillosis in beef cattle. American Journal of Veterinary Research. 15:5-14. 1954.

23. Jensen, Rue; W.E. Connell and A.W. Deem. Rumenitis and its relation to rate of ration and the proportion of concentrate in the ration of cattle. American Journal of Veterinary Research. 15:425-428. 1954.

24. Jensen, Rue; Paul R. Frey; Floyd Croce and W.E. Connell. Telangiectasis, "sawdust", and abscesses in the livers of beef cattle. Journal of the American Veterinary Medical Association. 110:256-261. 1947.

25. Jensen, Rue; Harold M. Deane; L. John Cooper; Victor A. Miller and W.E. Graham. The rumenitis liver abscess complex in beef cattle. American Journal of Veterinary Research. 15:202-216. 1954.

26. Kelly, Florene C. Bacteriology of artificially produced necrotic lesions in the oropharynx of the monkey. Journal of Infectious Diseases. 74:93-108. 1944.

27. McCullough, Norman B. Vitamin C and resistance of the guinea pig to infection with Bacterium necrophorum. Journal of Infectious Diseases. 62:34-53. 1938.

28. McVay, Leon V. Jr. and Douglas M. Sprunt. Bacteroides infections. Annals of Internal Medicine. 36:56-76. 1952.

29. Madin, Stewart H. A bacteriologic study of bovine liver abscesses. Veterinary Medicine. 44:248-251. 1949.

30. Matsushima, J. Personal communication. September, 1957.

31. Merchant, I.A. and R.A. Packer. Veterinary Bacteriology and Virology. 5th Edition. The Iowa State College Press, Ames, 1956.

32. Newsom, I.E. A bacteriologic study of liver abscesses in cattle. Journal of Infectious Diseases. 63:232-233. 1938.

33. Nolechek, W.F. Necrobacillosis in horses and mules. Journal of American Veterinary Medical Association. 54, New series 7:150-155. 1919.

34. Orcutt, Marion L. A study of Bacillus necrophorus obtained from cows. Journal of Bacteriology. 20:343-360. 1930.

35. Robinson, T.J.; E.E. Jasper and H.R. Guilbert. The isolation of Sphaerophorus necrophorus from the rumen together with some feed lot data on abscesses and telangiectasis. Journal of Animal Science. 10:733-741. 1951.

36. Rosen, M.N.; O.A. Brunetti, A.I. Beschoff, and J.A. Azevedo, Jr. An epizootic of foot rot in California deer. Transactions of the Sixteenth North American Wildlife Conference. 164-178. 1951.

37. Ryff, J.F. and A.M. Lee. The etiology of calf diphtheria. American Journal of Veterinary Research. 1:41-44. 1946.

38. Schrivaer, L.E. and A.M. Lee. The morphology, culture, isolation and immunity studies of Actinomyces necrophorus in calf diphtheria. Journal of the American Veterinary Medical Association. 85:360-376. 1934.

39. Shaw, Frederick W. Human necrobacillosis. Zentralblatt Fur Bakteriologie Parasitenkunde und Infektionskrankheiten. 132: 132-138. 1933.

40. Smith, Hilton A. Ulcerative lesions of the bovine rumen and their possible relation to hepatic abscesses. American Journal of Veterinary Research. 5:234-242. 1944.

41. Smith, Louis DS. Introduction to the Pathogenic Anaerobes. The University of Chicago Press, Chicago, 1955.

42. Turnicliff, E.A. A study of Actinomyces necrophorus in soil cultures. Journal of Infectious Diseases. 62:58-65. 1938.

43. West, Robert A. Jr; Keith H. Lewis and Walter E. Militzer. Growth of non-sporulating anaerobic bacteria of intestinal origin in synthetic media. Journal of Bacteriology. 43:165-169. 1942.

44. Wilson, G.S. and A.A. Miles. Topley and Wilson's Principles of Bacteriology and Immunity. 3rd. Edition. The Williams and Wilkins Company, Baltimore, 1946.