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An Organism Resembling Sphaerophorus necrophorus Isolated From a Beef Liver Abscess

James C. Canada

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AN ORGANISM RESEMBLING APHANOPHORUS NECROPHORUS
ISOLATED FROM A BEET 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 1968

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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.

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Joe
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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. Practically, the United States annually condemn 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, telangiectasia, 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 odorless, cheesy in appearance, and often under pressure. The second type of nonprocessable liver is due to telangiectasia. Jensen, Frey, Cross, and Connell (24) write, “Telangiectasia 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 telangiectasias and “sawdust” livers.
This investigation concerns an organism isolated from a beef liver abscess.

In addition, Wilson and Miles (44) offer the following synonyms: Schmorl's *bacillus*, Bang's *necrosis bacillus*, and *Fundiformis necrophorus*. Back and co-workers (8) add the synonyms, *Bacteirium necrophorus* and *Bacterium funduliforme*. One can readily visualize the difficulty that arises in securing information from the literature, concerning the liver abscesses organism, under such a taxonomic 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-
istice of *Sphaerophorus aggregatus*. Katsushima (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 aggregatus*. Orcutt (34) writes that the organism "...grows well on ordinary media plus a little serum." Schriner (38) disagrees and claims that this isolate is very difficult to grow.

Dack and co-workers (7) found that the colonies of *Sphaerophorus aggregatus* 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). Hewson (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 aggregatus*. Mannitol, sorbitol, arabinose, galactose, 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 Bergay (4) record that gelatin is not liquified, while Deck and co-workers (7) have found liquefaction in two of nine strains. Deck 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 Bergay (4) records that coagulation is present when the medium is inoculated with the organism. Reactions to both the methyl red and Voges-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). Deck 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 1927, 10.9 percent of all beef livers in Colorado were condemned because of
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, Nadia (39) found that 89.4 percent contained \textit{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 $2,601,355.30 (14).

\textit{Sphaerophorus necrophorus} has been known by its synonyms for many years. Demmensch 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). Schmerl named his isolate, *Erysipelas animalis*. While working with their isolate, Schmerl and an assistant developed lesions on their hands (39). This was the first time that *E. necrophorus* had been recorded from a human source. Veillon and Zuber, in 1897, worked with other pathogens of this genus (41). In 1898, Balle studied a bacillus, now thought to be *E. necrophorus*, associated with genital infections of men (40). Balle named the organism Bacillus funduliformis. *E. necrophorus* was first definitively isolated and cultured from a human source by Stemon 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 teeth while examining an abscess on the lip of the animal.

*E. 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.

Duck and co-workers (7) write that the organism is a common inhabitant of the intestinal tract of man, monkeys, and baboons. The organism finds favorable conditions for growth in ulcers of the intestines. Duck (6) also writes that the organism is probably a normal inhabitant of the mucous membranes of man and animals. Kelly (26), 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): "...salt diphtheria, necrotic ulcers of
the intestine in hog cholera, foot-rot in sheep and cattle, greased heel or necrotic scratches of horses, lip and leg ulceration of sheep, gas-
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*. Vitch (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, Haris (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
antelopes. 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 polymorpho-
nuclear leucocytes. The filamentous bacteria are found primarily in
the leucocyte zone within a capsular layer of connective tissue fiber.

Jenson, 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, meningitis, 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. Back and co-workers (7) claim that the bacterium finds favorable conditions in the intestine. Jeness 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 feed 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 food of infection may become established (23)."

This theory seems entirely feasible as Robinson, Jasper, and Quilbert (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 became infected by way of the umbilicus, 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 necrosus (39). Titres of 1:800 are found in the blood of normal cattle, horses, sheep, and swine (44). Back and co-workers (7) have found that strains of the organism are antigenically heterogeneous.

Smith (41) claims that immunisation 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 formalinised 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 intraperitoneally." Elder, Lee and Serivner (9) report, however, that they succeeded in preparing an antisus which would protect rabbits against experimental inoculations of the organism. Their work in immunising 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 pyogenes* variety aureus or *Escherichia coli*. Schriner and Lee (36) 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. Pitzer (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 scurvy had to be present in guinea pigs before there was a drop in resistance to *Sphaerophorus necrophorus*. In 1919, Neleschek (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 sulphonamide 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.
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 into 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 telangiectasia 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 repeated 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 odorless, 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 0.3 cubic centimeter of the cultures were inoculated into the chorion-allantoic fluid of the embryo. 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 thiglycollate survived. Attempts to recover the organisms from the infected chick embryos proved unsuccessful.

Laboratory mice were injected both subcutaneously and intraperitoneally with cultures of the organism grown in fluid thiglycollate. Mice were also injected with sterile fluid thiglycollate by both routes. All mice receiving the sterile fluid thiglycollate survived. The mice that were injected intraperitoneally with the cultures died after 34 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 thiglycollate 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 thiglycollate 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 anesthetised 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 thiglycollate.
Antibiotics

A preliminary experiment with the isolate in regard to antibiotics was initiated. The antibiotics used were penicillin, Aureosycin, bacitracin, neomycin, and tyrothricin. 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 tyrothricin. Growth was not observed in the tubes containing the penicillin, Aureosycin, 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 defibrinated. 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.

Toxins

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-
Mena was then precipitated; 0.3 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 cheesecloth. 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
- **K₂HPO₄**: 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
- **H₂O**: 1000 milliliters

#### "X" Medium

- **beef peptone**: 15 grams
- **yeast extract**: 5 grams
- **dextrose**: 5 grams
- **NaCl**: 2.5 grams
- **cysteine hydrochloride**: 0.6 gram
- **agar**: 0.75 gram
- **H₂O**: 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
- **H₂O**: 200 milliliters

#### "ZK" Medium

- **casitone**: 3 grams
- **dextrose**: 1 gram
- **NaCl**: 0.5 gram
- **L-cystine**: 0.1 gram
- **agar**: 0.15 gram
- **H₂O**: 200 milliliters
## "YY" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>3 grams</td>
</tr>
<tr>
<td>yeast extract</td>
<td>1 gram</td>
</tr>
<tr>
<td>dextrose</td>
<td>1 gram</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6 gram</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>200 milliliters</td>
</tr>
</tbody>
</table>

## "Z" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>beef peptone</td>
<td>3 grams</td>
</tr>
<tr>
<td>yeast extract</td>
<td>1 gram</td>
</tr>
<tr>
<td>dextrose</td>
<td>1 gram</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6 gram</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>200 milliliters</td>
</tr>
</tbody>
</table>

## "A" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin-free casein</td>
<td>3 grams</td>
</tr>
<tr>
<td>dextrose</td>
<td>1 gram</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6 gram</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>200 milliliters</td>
</tr>
</tbody>
</table>

## "B" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ten percent casein hydrolysate</td>
<td>30 milliliters</td>
</tr>
<tr>
<td>dextrose</td>
<td>1 gram</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6 gram</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>170 milliliters</td>
</tr>
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</table>

## "C" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>3 grams</td>
</tr>
<tr>
<td>dextrose</td>
<td>1 gram</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>200 milliliters</td>
</tr>
</tbody>
</table>

## "D" Medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>casein</td>
<td>3 grams</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5 gram</td>
</tr>
<tr>
<td>agar</td>
<td>0.15 gram</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>0.1 gram</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>200 milliliters</td>
</tr>
</tbody>
</table>
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 aerocaphorus (Plagge, 1886) Pevrot, 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


