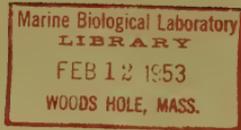


# FURUNCULOSIS OF FISH



SPECIAL SCIENTIFIC REPORT: FISHERIES No. 84

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FISH AND WILDLIFE SERVICE

## Explanatory Note

The series embodies results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. It is issued in limited quantities for the official use of Federal, State or cooperating Agencies and in processed form for economy and to avoid delay in publication.

This paper was originally submitted as a Master's Thesis. Dr. S. F. Snieszko of the Service's Microbiological Laboratory at Leetown, West Virginia, obtained a copy of the manuscript and recommended that it be published so that this valuable material would be available to all fishery workers. The author, unable to find a publisher in Canada, agreed to the Fish and Wildlife Service's publication in this series. This paper is based upon information gathered from the literature and all references on furunculosis of fish have been consulted.

Washington, D. C.  
December, 1952

United States Department of the Interior, Oscar L. Chapman, Secretary,  
Fish and Wildlife Service, Albert M. Day, Director

FURUNCULOSIS OF FISH

By Bruce M. McCraw  
Department of Parasitology, Ontario Veterinary College  
Guelph, Canada

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## Furunculosis of Fish

No fish disease is to be feared more by the hatchery man than furunculosis, or the "great red plague" of salmon and trout. In 1935, Fish stated that this disease had spread throughout regions of the United States and Canada to such a degree that it threatened our entire system of artificial propagation of salmon and trout. The shipping of infected hatchery fish has established this disease in many American trout and salmon hatcheries. Of still greater importance is the possibility that furunculosis may become well established among wild trout through planting of infected fish.

Although furunculosis in wild trout of North America has by no means assumed epidemic proportions, in Great Britain this disease became so serious that a committee was formed to study ways of combating it (Furunculosis Committee, 1930, 1933, 1935). In two rivers of Great Britain, which were by no means first class, the losses over periods of 3 and 6 years were \$6,000 and \$3,600. In other rivers these figures were even exceeded (Furunculosis Committee 1933).

Furunculosis was originally described from hatchery fish in Germany by Emmerich and Weibel (1894), who found the causative agent to be a bacterium which they named Bacterium salmonicida. After their description, the disease was commonly recognized in German trout hatcheries where it was believed to be strictly a hatchery disease (Fish 1937). Plehn dispelled this belief in 1909 by finding furunculosis among wild fish in the Province of Bavaria. Extensive studies by this author in 1909 and 1911 revealed the existence of the disease in wild trout taken from 25 rivers and streams of that Province. Shortly after this, furunculosis was found in other parts of Germany, in France, Austria, Belgium, and Switzerland (Fuhrman 1909, Pittet 1910, Surbeck 1911). The disease was first observed in Great Britain among mature salmon from four rivers (Masterman and Arkwright 1911). Three years later it was found among salmon in Ireland by Mejtam (1914). In recent years an epizootic disease occurred among trout farms in Japan which was thought to be furunculosis (Furunculosis Committee 1933).

The first definite proof that furunculosis existed in the Western Hemisphere was offered by Marsh (1902) who found it to be the cause of an epizootic among hatchery fish in Michigan. It has since been found in many salmon and trout hatcheries throughout the United States (Fish 1937), and Smith (1942) estimated that it has occurred in more than one-fourth of the United States.

Up to 1937, furunculosis had not been reported from wild fish in the United States (Fish 1937). In 1937 furunculosis was described in adult Loch Leven trout (Salmo trutta levensis) in Wyoming under circumstances indicating that it was not merely a hatchery epidemic transplanted to open waters, but the true establishment of the disease among wild stock (Fish 1937).

It is generally believed that furunculosis was brought to the Western Hemisphere with brown trout introduced into the United States from Germany. However, Fish (1937) feels it is far more likely that furunculosis was originally a disease of rainbow trout (Salmo gairdnerii) of the Western United States. His conclusion is based upon the fact that since rainbow trout in the United States possess a relatively high immunity to furunculosis, the disease must be one of long standing.

Turning to Canada, furunculosis has been reported by Duff and Stewart (1933) from wild Rocky Mountain whitefish (Prosopium williamsoni), Dolly Varden (Salvelinus malma spectabilis), and cutthroat trout (Salmo clarkii) of Elk River in southeastern British Columbia.

#### RECORDS OF BACTERIAL DISEASES OF FISH BEFORE 1894

Although furunculosis was not described until 1894, there exists the possibility that some evidence of it may be found in earlier records of fish diseases. Williamson (1929) reviewed the literature with this in mind but found it impossible to identify with certainty the bacteria described in older records. The general nature of the infections described in the earlier literature was, however, easily recognized.

From 1866 to 1868 an epizootic disease among perch in Lake Geneva was investigated by Forel (1868) and Forel and du Plessis (1866-67). The fish died in very large numbers and the investigators referred to the disease as "typhus" or "typhoid" of fish, apparently because one of the symptoms was a yellow discharge from the cloaca. The condition was that of a general infection, in which the bacteria appeared in the blood during life. Small superficial hemorrhages were seen and there were degenerative changes in muscle, skin, and fins. From the description, a secondary infection, possibly the same as that now recognized as Saprolegnia ferax (Hume Patterson 1903) appeared to have taken place, as patches of white mossy substance were seen on the skin. Hemolysis took place in the blood vessels, but the bacteria disappeared rapidly from the blood after death. The organisms seen were short, spindle-shaped rods, often in pairs. The mode of infection was not discovered. Healthy fish kept in tanks beside diseased fish did not become infected.

Fabre-Domergue (1890) described a tumor-like lesion in Caranx trachurus and stated that the lesion was full of bacteria. The organisms were often seen in pairs, and larger forms were twisted spirally. He made cultures on fish gelatin which was liquefied and assumed a green fluorescence. The bacteria were, he thought, Gram-negative. Experimental inoculation of cultures into several fish was unsuccessful. Williamson (1929) felt that Fabre-Domergue (1890) had isolated Pseudomonas fluorescens, although not in pure culture, and that the spiral organism present was either a water vibrio or spirillum. She also felt that the lesion was a connective tissue tumor which had undergone degeneration and had been invaded by water organisms.

From 1892 for a few years great attention was given to epidemics of bacterial diseases of fish on the Continent. Fischel and Enoch (1892) investigated a disease of carp and isolated an organism which they state was a Gram-negative bacillus, formed spores, and produced a toxic substance for both cold-blooded and warm-blooded animals.

Charrin (1893) isolated several bacilli, one of which proved pathogenic, from diseased fish on the Rhone. This pathogen was a motile bacillus which grew at 20° C. but not at 37° C. It liquefied gelatin and produced acid and clot in milk. Fish became infected from water contaminated with the bacillus, and hemorrhages occurred in muscle and skin. Toxins were produced and these were precipitable by alcohol and were virulent for warm-blooded animals as well as for fish.

Bataillon (1893-94) and Bataillon and Dubard (1893) investigated a disease affecting trout and their eggs, and also crayfish and frogs. The organism described was a motile bacillus which often occurred in pairs. It liquefied gelatin which became slightly green but not fluorescent. The fish affected showed intramuscular lesions, general congestion and blood infection. The strains isolated from trout, frogs, and crayfish, all had the same characteristics and were pathogenic for these animals. Infection occurred from contaminated water and a toxin precipitable by alcohol was formed. Bataillon (1893-94) thought that this organism belonged to the group "Termo" (Proteus). Williamson (1929) felt that the bacillus isolated by Charrin (1893) was the same as this.

In Russia, Sieber-Schoumowa (1895) described another toxin-producing organism from diseased fish and called it B. piscicidus agilis. She stated that it was Gram-negative and produced spores. Death was caused in experimental animals by oral administration of either the bacillus or its toxin.

Several cases have been recorded of disease production in fish by Vibrios. Canestrini (1892-93) reported a disease of eels caused by an organism like V. comma. This vibrio was pathogenic for fish and frogs, but not for warm-blooded animals. It retained its viability longer in salt water than in fresh water.

## DESCRIPTION OF CAUSATIVE ORGANISM

### Classification

Bacterium salmonicida is classified as follows according to Bergey's Manual of Determinative Bacteriology (Breed et al. 1948).

Kingdom Plant  
Phylum Thallophyta  
Class Schizomycetes Nägeli

Plants of this Class are typically unicellular. The cells are usually small, sometimes ultramicroscopic, and frequently motile. They lack a definitely organized nucleus. Individual cells may be spherical; or straight, curved, or spiral rods.

### Order I. Eubacteriales Buchanan

Organisms of this Order are rigid. The cells occur singly, in chains, or in masses. They are neither branching or mycelial in character, nor arranged in filaments. They are not acidfast and motility when present is by means of flagella.

### Suborder I. Eubacteriineae

Organisms of this suborder do not possess photosynthetic pigments and do not contain free sulphur. They are not attached by a stalk and do not deposit ferric hydroxide.

### Family XII. Bacteriaceae Cohn

#### Genus and Species

Bacterium salmonicida Lehmann and Neumann

### Morphology and Staining

Bacterium salmonicida is in general a short cylindrical organism (Emmerich and Weibel 1894, Plehn 1924, Williamson 1928, Duff and Stewart 1933), nonmotile and non-spore-forming (Emmerich and Weibel 1894, Duff and Stewart 1933), with a tendency toward pleomorphism (Marsh 1902, 1903, Arkwright 1912, Mettam 1914). It often occurs in pairs. Williamson (1928) gives its dimensions as 1 to 4  $\mu$  in length by 0.8 to 1  $\mu$  in width but smaller and larger forms have been reported (Marsh 1903).

According to Duff and Stewart (1933) in very young agar cultures nearly all cells are coccoid to ovoid, longer forms appearing in cultures after 24 hours. In blood and lesions of the host, Marsh (1903) found B. salmonicida generally larger and more bacillary than when growing on artificial media. A capsule has not been demonstrated.

B. salmonicida is Gram-negative (Emmerich and Weibel 1894 and others), tending to stain unevenly (Marsh 1903, Williamson 1928) and often showing bipolar staining (Williamson 1928), Duff and Stewart 1933). Marsh (1903) found that methylene blue and thionin gave excellent results.

### Viability

#### Temperature, light and drying

The temperature range for growth of B. salmonicida is 5° to 30° or 32° C. (Arkwright 1912, Williamson 1928, Duff and Stewart 1933, Furunculosis Committee 1935). By some authors the optimum is stated to be between 20° and 22° C. (Marsh 1903, Williamson 1928, Furunculosis Committee 1930) but Emmerich and Weibel (1894) and Davis (1946), claim it to be between 10° and 15° C. These differences in findings could be projected upon (a) variations amongst strains, or (b) variations in media used. Arkwright (1912) found that cultures remained alive at 1° C. for at least 4 months, and that at 35° or 36° C. the organism will sometimes reproduce to a slight extent. Generally at 37° C. either no growth occurs (Emmerich and Weibel 1894) or cultures soon die (Arkwright 1912, Mettam 1914, Williamson 1928) but Duff and Stewart (1933) accustomed a strain to give growth and pigment at this temperature. Williamson (1928) and Duncan (1932) found that a broth culture of the organism was killed in one minute at 60° C. Emmerich and Weibel (1894) also found that 60° C. rapidly killed the organism. Plehn (1911) stated that 40° C. was sufficient to kill it.

According to Duncan (1932) bright daylight kills the organism. Williamson (1928) showed that B. salmonicida died when dried for 6 hours and Duncan (1932) found that on drying a drop of broth culture at room temperature, the organism did not develop after 24 hours' culturing.

#### In water

Various authors have recorded that B. salmonicida is recovered from distilled water from 4 days to 2 weeks. In unsterilized distilled water Williamson (1928) recovered the organism up until 4 days and in sterilized distilled water up until 5 days. Duncan (1932) found the organism to be alive at the end of a week in distilled water and Horne (1928) gave a figure of 14 days for survival in distilled water.

B. salmonicida is recoverable from tap water up to 2 to 5 days. Plehn (1924) claims that pure water is harmful to the organism, and she could not recover it in pure town water (Munich) after 2 days. Table 1 gives the findings of various authorities in tap water.

In unsterilized natural (river) water, Williamson (1928) recovered the organism up to 2 days and in sterilized natural water up to 5 days in very scanty numbers. In natural waters containing many organisms, Duncan (1932) found that the bacterium died out in 24 to 30 hours.

B. salmonicida survives from a few hours to 3 days in sea water. In unsterilized sea water Williamson (1928) recovered the organism up to 2 days and in sterilized sea water up to 3 days. Arkwright (1912) recorded no growth after 19 hours in sterilized sea water and Duncan (1932) after 24 to 30 hours in plain sea water. With mixtures of sea water and tap water in equal parts, Arkwright (1912) noted that none of the bacteria could be recovered after 19 hours; but from a mixture containing sea water 25 percent and tap water 75 percent, a fair growth was obtained after 45 hours but none after 67 hours. Arkwright (1912) also found that salt water (3 percent sodium chloride in distilled water) had a destructive effect on the bacteria. Growth from agar plate was emulsified in 500 cc. of this salt solution, and one drop taken after 2 days gave only one colony on an agar plate.

Table 1.--Viability of B. salmonicida in tap water

<u>Authority</u>	<u>Date</u>	<u>Results</u>
Arkwright	1912	From sterile tap water the organism could be recovered alive in diminished numbers after 5 days.
Plehn	1924	Organism was recovered from pure town water (Munich) after 2 days but not after 3 days.
Herne	1928	<u>B. salmonicida</u> persisted in tap water for 3 to 4 days.
Williamson	1928	Organism was recovered from both sterilized and unsterilized tap water after 3 days in very scanty numbers.
Duncan	1932	In tap water containing other microorganisms, living <u>B. salmonicida</u> were not detected after 4 days.

## In sewage

Duff and associates (1940) made extensive studies on the effect of polluted water on the viability of B. salmonicida. Briefly, their methods were as follows: experimental containers consisted of 4-liter florence flasks, each containing 2 liters of fluid. Samples withdrawn for estimation of bacterial numbers were always at least 1 cc. in volume, and were taken after thorough mixing of the contents of the container. Where necessary, decimal dilutions of such samples were made, and from such dilutions triplicate platings were carried out in most instances. In cases where large numbers of bacteria were present, only those plates were used for count estimations which contained more than 40 and fewer than 200 colonies per plate. Inocula consisted of standardized suspensions invariably made from 24-hour agar slant cultures of B. salmonicida.

A known volume of sample or of sample dilution (usually 1 cc.) was placed in the center of a previously poured and dried plate of ordinary nutrient agar. By means of a sterile glass spreader, and by rolling, the entire surface was uniformly wetted with the inoculum. About 2 hours with tilted lid at 26° C. were allowed for absorption and evaporation before finally inverting the plate. Incubation was carried on at 22 ± ° C. A differential medium was developed which served to distinguish B. salmonicida from most sewage microflora.

All sewage used in the experiments of Duff and associates (1940) was known to be free from trade and other chemical wastes. The actual "sewage" placed in experimental flasks consisted of decanted supernatant fluid from fresh sewage which had stood 2 hours in the laboratory.

### Undiluted Fresh Sewage

Using an initial inoculum of 350 ± 50 B. salmonicida per cc., Duff and associates (1940) noted an increase in 24 hours of well over 2,000 per cc. The figure of "over 2,000 per cc." was a minimum figure based on having picked from two of three plates of a 1:1,000 sample dilution, two and four colonies, respectively, whose identity was subsequently confirmed by morphological and cultural methods. Counts of similar magnitude were obtained for 15 days, after which time increase of sewage flora in experimental vessels rendered further identification impossible.

In another experiment the original bacterial content was partly destroyed by heating at 56° C. for 2 hours. Sewage count was thus reduced from 2,750 per cc. to about 130 per cc. The inoculum consisted of 100 ± 50 B. salmonicida per cc. Again a definite multiplication followed, with a count of 200,000 B. salmonicida per cc. at the end of the third day. Confirmed B. salmonicida colonies were recovered up to the twenty-third day.

Plehn (1924) found that B. salmonicida in polluted water taken from below Munich increased from 5,800 per cc. at the beginning of the experiment to 8,803,000 per cc. at the end of 3 days.

Preparing emulsions of B. salmonicida in 15 cc. of highly polluted drain water, Williamson (1928) found that when it was unsterilized the organism could not be recovered, but when sterilized, B. salmonicida persisted for 3 days, though in scanty numbers.

Similarly, Horne (1928) found that when 1 cc. of broth culture was sown into 100 cc. of crude sewage and sewage effluent and incubated at room temperature, B. salmonicida was not recovered after 2 days. Sewage was examined for the presence of B. salmonicida by sowing a loopful onto an agar plate.

The results of Duff and associates (1940) thus support the findings of Plehn (1924) as to the ability of B. salmonicida to multiply in sewage-polluted waters but are contrary to those of Horne (1928) and Williamson (1928). Duff and associates (1940) point out that the results of the latter workers may have been influenced by the presence of inhibitory trade wastes in sewage samples employed since the absence of such agents is not explicitly recorded in their reports. The disagreement may also be accounted for by differences in methods of sampling employed. Duff and associates (1940) found that at least in diluted autoclaved sewage (see below) of widely varying organic content B. salmonicida count may drop to a very low figure (well below 100 per cc.) within the first 10 days before extensive multiplication sets in. Duff and associates (1940) also point out that whereas Horne (1928) and Williamson (1928) both sampled by means of the loop, and they by withdrawing 1 cc. of sample, the employment of such small samples may have led to the lack of recovery of the bacterium at a stage where it may have been present in very small numbers. There are approximately 75 loopfuls in 1 cc. of B. salmonicida culture or in 1 cc. of average sewage dilution.

The answer to the question of the viability of B. salmonicida in polluted water in nature may reside in the nature of the polluting material. There is no definite evidence of B. salmonicida being capable of multiplying or surviving for any length of time in noxious trade wastes. On the other hand, the extensive work of Duff and associates (1940) has shown that, providing sewage is free from trade and other chemical wastes, B. salmonicida will survive under conditions of the laboratory for a considerable period (23 days). As far back as 1924, Plehn stated that the danger of furunculosis increases according to the quantity of pollution by organic matter, and she mentioned effluents from dairies as being particularly harmful.

#### Undiluted Sewage Sterilized by Berkefeld Filtration

Duff and associates (1940) found that an initial inoculum of 100 ± 50 B. salmonicida per cc. first greatly multiplied, then decreased, the last colony appearing on the thirteenth day, when estimating the number of survivors by plate method.

### Autoclaved Sewage

Again, with an initial inoculum of  $100 \pm 50$ , Duff and associates (1940) noted definite multiplication (between the first and fourth day) recovering the last colony on the twenty-second day. A second experiment gave similar results, except that viable organisms were recovered up to the thirty-fifth day.

Experiments were carried out by Duff and associates (1940) with varying dilutions of autoclaved sewage supernatant. An inoculum of  $350 \pm 50$  B. salmonicida per cc. was planted into 2-liter amounts of decimal dilutions of sewage supernatant in tap water. In all cases the numbers of B. salmonicida after first dropping below 100 per cc. within the first 10 days, quickly multiplied to over 200,000,000 per cc., remaining greatly in excess of 2,000 per cc. for periods of 23 to 29 days, when the numbers dropped below 2,000 per cc. The experiment was terminated on the thirtieth day with a B. salmonicida population of over 1,000 per cc. still in existence.

Commenting on their results, Duff and associates (1940) pointed out that it is well known that simply confining a fluid containing a bacterial population in laboratory glassware may lead to a multiplication of the bacteria per unit volume (Winslow 1928, Waksman and Carey 1935, Zobell and Anderson 1936). They felt that if one could consider that the addition of a B. salmonicida population to a sterile sewage suspension meant merely the addition of an inoculum to a relatively poor nutrient medium, there would be no need for further explanation. But their results indicated that a very similar multiplication occurs in widely varying dilutions of sewage. Multiplication of B. salmonicida to over 200,000,000 per cc. occurred between 9 and 16 days in dilutions of 1:1,000 to 1:1,000,000 suggesting that dilution of nutrient is not a major factor, although when nutrient is completely eliminated (as in tap water control) no multiplication is possible. It is therefore possible that some of the multiplication of the pathogen in their experiments may be attributable to confinement of its menstruum in laboratory glassware. Should this be the case, such findings might have no direct bearing on the behavior of the pathogen under field conditions in which the organism is released into practically an infinite volume of fluid.

### Ether-Treated Sewage

Duff and associates (1940) also studied the behavior of B. salmonicida in ether-treated sewage. In this experiment a portion of the original living sewage microflora was retained alive in sewage. This result was effected by exposing sewage to ether vapor as follows. Two liters of fluid were placed in a 4-liter flask, closed tightly with a rubber stopper. From the stopper was suspended a wide vial containing 30 cc. of ether. The flask was shaken frequently, so as not to spill

the liquid ether. When the ether had completely disappeared from the vial, a sterile cotton plug was substituted for the rubber bung. The flask was then allowed to stand at 37° C. until the odor of ether was no longer perceptible. This procedure resulted in the destruction of 60 to 75 percent of the original microflora.

After adding an initial inoculum of 2,000 ± 200 B. salmonicida per cc. to ether-treated undiluted sewage, rapid multiplication of the bacterium occurred throughout the first 2 days, at the end of which time numbers amounted to 700,000 per cc., or an increase of 350-fold the original inoculum. Multiplication continued to a still higher degree, and by about the fifth day B. salmonicida colonies (on agar plates) were uncountable. On the seventh day numbers were 1,000,000 per cc. and a subsequent minor fluctuation was followed by a fairly rapid drop over a period of 6 days when the count was reduced to the neighborhood of only 70 per cc. A second sharp rise occurred over the succeeding 9 days, finally followed by a gradual and irregular decrease in numbers over 43 days. The last recovery of B. salmonicida occurred on the sixty-seventh day after inoculation. During the final gradual decrease, a general increase in numbers of native sewage colonies was noted, although counts were not made (Duff et al. 1940).

#### Saprophytic Existence and Survival of B. salmonicida in Dead Fish

Evidence was obtained by the Furunculosis Committee (1933) that B. salmonicida survives in the furuncles of dead fish and is still virulent after 6 days at a temperature of about 40° F. (5° C.).

The results of viability studies show conclusively that B. salmonicida is an obligate parasite with no power of existing as a saprophyte under natural conditions. Although it has been found to survive up to 67 days in ether-treated sewage (Duff et al. 1940), its existence in river and tap water is short, a few days at the most.

#### Cultural Characters

##### Relation to free oxygen

B. salmonicida is aerobic and facultatively anaerobic (Williamson 1928, Duff and Stewart 1933). Pigment production does not occur anaerobically (Williamson 1928).

##### Agar

Growth on agar media is good. Emmerich and Weibel (1894) found that at first growth on agar was whitish gray, later becoming yellowish. After some weeks, however, cultures became brownish. Although Plehn

(1909) noted scanty growth with involution forms on agar, her results of that year have not been generally upheld. She also claimed that pigment production on agar occurred later and was much less pronounced than on other media (Plehn 1911).

Williamson (1928) found a delicate growth of small transparent colonies on agar plate in 24 hours at 15° to 20° C. In 7 days colonies were on the average 1 mm. in diameter, circular, raised, slightly brownish in color, semi-opaque, moist and glistening. Later pigment diffused into the medium which became dark brown. Growth similar to that on agar plate occurred on agar slant.

Duff and Stewart (1933) found that B. salmonicida grew well on peptone meat-extract agar with fairly wide range of pH (6.0 to 8.0) for good growth. A whitish, effuse, glistening, convex, translucent growth appeared in 2 days. Growth later became abundant and brownish gray, the medium becoming brown. In 3 to 6 days pigment becomes coffee brown (Arkwright 1912, Duff and Stewart 1933).

On serum agar Williamson (1928) found growth exceptionally abundant with intense pigment production.

Fairly good growth occurs on 0.5 percent bile-salt agar according to Arkwright (1912).

On blood agar hemolysis rapidly occurs (Williamson, 1928). Duff and Stewart (1933) noted hemolysis in 2 to 3 days on rabbit blood agar plates, the colonies becoming greenish about the seventh day. Stevens and Keil (1931) isolated a strain of B. salmonicida which showed no hemolysis after 14 days but such a finding is exceptional.

#### Broth

A fine flocculent growth appears throughout the medium with a sediment upon the sides and bottom of the tube (Williamson 1928, Duff and Stewart 1933). A marked growth is visible after 18 to 24 hours (Marsh 1903, Stevens and Keil 1931). Marsh (1903) claimed that a delicate pellicle might form after 5 days. The appearance of pigment in broth is variable. Stevens and Keil (1931) found it after the third day; Williamson (1928) stated that she observed it only after 2 months; and Mettam (1914) records that very little pigment is produced in broth. According to Duff and Stewart (1933) a brown ring of pigment forms at the top of a broth tube in 10 to 20 days, which gradually diffuses throughout the medium.

Arkwright (1912) found that in fish broth growth was vigorous but that pigment production was poor in peptone beef broth.

## Potato

At no time is growth profuse on potato (Marsh 1903, Arkwright 1912, Mettam 1914, Williamson 1928). Duff and Stewart (1933) found a scant, creamy, raised filiform growth in about 5 days which later became a dirty grayish color. Stevens and Keil (1931) claimed a slight growth occurred after 48 hours and pigment after the third day.

### Pigment production

Several authors have studied the brown pigment produced by B. salmonicida in culture as this is one of the chief characters by which the organism may be identified. Chromogenesis has been found to occur only under aerobic conditions (Williamson 1928, Duff and Stewart 1933).

It has been found that a slight acidity of media tends to inhibit pigment production (Plehn 1911, Williamson 1928). Williamson (1928) showed that when the pH was less than 7.6 chromogenesis was proportionally less. Similarly, Marsh (1903) found that pigment production was inhibited in extremes of reaction but did occur in alkaline, neutral and acid media.

The optimum temperature for chromogenesis is the optimum for growth (Furunculosis Committee 1930). Marsh (1903) stated that higher temperatures inhibited pigment production faster than growth of the organism.

Williamson (1928) found chromogenesis to be at a maximum on solid media, appearing on fluid media after a longer time. It occurred in 1 to 2 months in liquefied gelatin (Williamson 1928). In broth, pigment has been reported after 3 days to 2 months (Stevens and Keil 1931, Williamson 1928). Marsh (1903) claimed that no pigment formed on gelatin but Plehn (1911) found it after the second week.

The intensity of pigment produced by different strains of the organism is variable (Duff and Stewart 1933). Occasionally after prolonged subculture a strain may lose its ability to produce pigment on agar only to regain this property after further subculture or following a change to serum medium. Arkwright (1912) found that pigment may not be formed on primary isolation of the organism but would show up on subculture. Again, sometimes he noted that plates which were crowded with almost pure cultures of B. salmonicida remained without pigment for 5 days or more.

Growth but lack of pigment production does not necessarily indicate that B. salmonicida is absent. This is due to the fact that various factors (pH, temperature, nature of media, and strain of organism) may determine the time of appearance, and even the presence or absence, of pigmentation.

According to Duff and Stewart (1933) the pigment is water-soluble. If ethyl alcohol is added to an agar slant the pigment diffuses out into the solution (Arkwright 1912, Duff and Stewart 1933). Arkwright (1912) demonstrated from his studies that when dried the pigment was not soluble in absolute alcohol.

An experiment performed by Williamson (1928) showed that the coloration produced by B. salmonicida is a true pigment--both active growth and free oxygen being necessary--and not an oxidation product. Three cultures (Nos. 1, 2, and 3) of the organism were put up at the same time on fish-extract-agar slopes. All were incubated at room temperature, No. 1 aerobically, Nos. 2 and 3 anaerobically, in Buchner tubes. In 4 days pigment began to appear in No. 1, and all were kept for 14 days, when there was intense pigmentation in No. 1 and no trace of pigment in No. 2 or No. 3. Then No. 2 was removed from the Buchner tube and left exposed to air, while No. 3 on removal from the Buchner tube, was sterilized by exposure to 60° C. for 1 hour. On the same day a fresh culture, No. 4, was made and kept under aerobic conditions. Four days later Nos. 2 and 4 showed commencing pigment production, and in 6 days this was marked; No. 3 remained unpigmented.

#### Salt stability

B. salmonicida has been found to be auto-agglutinable in salt solutions of as low concentration as 0.1 percent sodium chloride (Arkwright 1912, Williamson 1928). However, Duff (1939), while dealing with special phases of the organism, consistently, obtained stable suspensions of all pure S- (smooth) and G-phase cultures in 0.8 percent sodium chloride (see below). He also found that R-phase (rough) cultures, while readily auto-agglutinable in salines down to 0.2 percent, formed satisfactorily stable suspensions in 0.05 percent sodium chloride (see below). In general it may be said that B. salmonicida is auto-agglutinable in salt solutions of very low concentration.

Emulsions in distilled water are unstable according to Williamson (1928). Arkwright (1912) did find, however, that an emulsion remained uniform for 24 hours.

#### Biochemical Reactions

##### Gelatin

The findings of various workers on the reaction of B. salmonicida to gelatin are quite variable. Table 2 lists the results of various authorities with respect to growth, liquefaction, and pigment production.

Table 2 indicates that in general B. salmonicida grows rapidly on gelatin with liquefaction occurring in most cases, from 24 hours to a few days. Pigment is relatively common, being recorded after the third day to 20 days. Gas is reported in only two cases. The shape of the train of liquefaction is variably reported as crateriform, infundibular form, stratiform, and sacculate.

#### Serum media

Table 3 shows the results of various authorities on the reaction of B. salmonicida on solidified serum with respect to growth, liquefaction and pigment production.

Table 3 shows that B. salmonicida grows rapidly on solidified serum with liquefaction occurring from the second to fourteenth day. Pigment production also occurs, variably from the third to fourteenth day.

#### Milk

Table 4 shows that in general B. salmonicida grows in milk, sometimes abundantly; brings about peptonization of milk with acid production; does not coagulate milk. Pigment production occurs in about 20 days as in the case of Duff and Stewart (1933), but it is not a prominent feature as five authorities make no mention of it. Stevens and Keil (1931) record no change whatsoever in milk.

#### Carbohydrate reactions

Table 5 summarizes the carbohydrate reactions of B. salmonicida.

Table 5 shows that in general glucose and mannite are fermented and that sucrose, lactose, and dulcitol are not fermented. In addition Mettam (1914), Duff and Stewart (1933) found that galactose, levulose, maltose, and salicin were also fermented but that raffinose was not.

#### Other biochemical reactions

According to Duff and Stewart (1933), starch is utilized in Eckford's broth. An initial pH of 7.0 is reduced to 6.0 in 2 days and to 5.4 in 9 days. It is also utilized in starch agar.

Nitrates are reduced to nitrites in 5 to 7 days (Marsh 1903, Duff and Stewart 1933). According to Marsh (1903) 7-day cultures contain both nitrites and ammonia. He also found that 40-day cultures contain no nitrite but give a strong test for ammonia.

Table 2.—Action of B. salmonicida on gelatin with respect to growth, liquefaction, gas and pigment production

Authority	Growth	Liquefaction & gas production	Pigment production
Emmerich and Weibel (1894) after Arkwright 1912).	Slow, very small colonies appeared during first 2 or 3 days.	Later liquefaction with gas production.	No mention.
Marsh (1902).	Abundant growth.	Liquefaction abundant; crateriform infundibular form or stratiform.	No mention.
Plehn (1909) (after Arkwright 1912).	Growth.	No mention.	Culture became slightly brown in 10 days and coffee brown in 3 weeks.
Plehn (1911) (after Arkwright 1912).	Growth.	No mention.	Pigment after 2 weeks; most marked near surface of culture.
Arkwright (1912).	Organism grew well.	Liquefaction within 24 or 48 hours, infundibular form. Gas in most cases.	Pigment not usually formed.
Mettam (1914).	Surface cultures on gelatin slant caused pitting of surface of medium.	Rapid liquefaction.	No pigment.
Williamson (1928).	Rapid growth.	Liquefaction in 24 hours from below upwards.	Later, upper part of medium became brown.
Stevens and Keil (1931).	Rapid growth; flocculent in liquefied medium.	After 48 hours upper part of medium was liquefied. Complete liquefaction.	Pigment near surface of liquefied medium after third day.
Duff and Stewart (1933).	Growth.	Liquefaction in 3 to 8 days; saculate or infundibular form. In some cases liquefaction began at bottom of stab, but this was not a constant feature.	A brown ring of pigment was formed at top in 20 days.

Table 3.--Action of B. salmonicida on solidified serum with respect to growth, liquefaction, gas and pigment production

Authority	Growth	Liquefaction and gas production	Pigment production
Marsh (1903).	Visible growth in 18 hours. After 11 days it became slow.	Evident liquefaction on second day.	A marked brown color after 3 to 4 days. Dark brown after about 11 days.
Arkwright (1912).	Grew readily.	Slow liquefaction in 7 to 14 days.	Medium became deep brown in 7 to 14 days.
Mettam (1914).	Grew readily.	Liquefaction.	Pigment production.
Williamson (1928).	Growth.	Slow liquefaction on Loeffler's serum.	Pigment production.
Stevens and Keil (1931).	Rich growth in 24 hours.	After seventh day gradual liquefaction on Loeffler's serum.	Pigment on sixth day.
Duff and Stewart (1933).	Growth.	Complete liquefaction in 6 to 10 days. Gas sometimes noted.	Much pigment.

Table 4.--Results of various authorities on the action of B. salmonicida in milk

Authority	Growth	Peptonization	Coagulation	Production of acid	Production of pigment
Marsh (1903)	Abundant	+	-	Slight or unchanged	No mention
Arkwright (1912)	Growth	+	-	+	No mention
Mettam (1914)	Grew without difficulty	No mention	-	No mention	+
Williamson (1928)	Growth	+ Slow peptonization	-	+	No mention
Stevens and Keil (1931)	No mention	No mention	-	-	No mention
Duncan (1932)	Growth	+ Slow peptonization	-	+	No mention
Duff and Stewart (1933)	Growth	+ In about 13 days	Most strains coagulate milk in about 7 days without acid production; occasionally slight acid without coagulation		Pigment in about 20 days

Table 5.--Action of *B. salmonicida* on various carbohydrates

Sugar	Emmerich and Weibel* (1894)	Marsh (1903)	Arkwright (1912)	Mettam (1914)	Williamson (1928)	Furunculosis Committee (1930)
Arabinose	0	0	0	0	0	0
Xylose	0	0	0	0	0	0
Glucose	0	A $\pm$	A G	A	A G	A;G $\pm$
Galactose	0	0	0	A	0	0
Levulose	0	0	0	A	0	0
Mannite	0	0	A G	A	A G	A;G $\pm$
Sucrose	-	A $\pm$	Alk	-	Alk Pigm	-
Lactose	0	A $\pm$	Alk Pigm	?	Alk Pigm	-
Maltose	0	0	0	A	0	0
Raffinose	0	0	0	-	0	0
Salicin	0	0	0	A	0	0
Inulin	0	0	0	?	0	0
Dulcitate	0	0	Alk	0	Alk Pigm	-

\*After Arkwright (1912)

A = acid; G = gas; Alk = medium alkalinized; Pigm = brown pigment produced;

-- = negative; 0 = reaction in carbohydrate not studied.

Indole is not produced (Marsh 1903, Arkwright 1912, Mettam 1914, Williamson 1928, Furunculosis Committee 1930, Duncan 1932, Duff and Stewart 1933).

Growth occurs in peptone water (Marsh 1903, Arkwright 1912, Williamson 1928). Marsh (1903) stated that growth resembled that in broth but proceeded more slowly. Arkwright (1912) claimed that pigment was produced more readily than in broth.

#### Agglutination of *B. salmonicida* with Fish Antisera

Although Arkwright (1912) was unsuccessful, Duff (1942) while making a study of the oral immunization of trout obtained good results. The antigen was suspended in 0.05 percent sodium chloride solution instead of the common 0.85 percent. Incubation was carried out for 4 hours at 37° C., followed by ice-box storage overnight. Preliminary tests with immune rabbit-sera specific for *B. salmonicida* showed the most sensitive bacterial suspension to consist of a 48-hour agar growth washed off with 0.05 percent saline solution. The suspension was not killed and was diluted to No. 4 McFarland turbidity standard for use. Duff (1942) found that if kept in the cold the antigen is sensitive up to 48 hours, after which sensitivity decreases. Blood was obtained from trout by heart-puncture. A small quantity of heparin injected directly into the heart was found necessary to prevent clotting in the fine needles. His results are shown in table 6.

#### *B. salmonicida* Complement Fixation

Blake and Anderson (1930) submitted 82 strains of *B. salmonicida* to the complement fixation test including 60 isolated from fish in England and Scotland, and 22 received from other countries (Austria, Germany, Irish Free State, United States). All gave strongly positive reactions. Twenty-one organisms other than *B. salmonicida*, isolated from water, gave consistently negative results. Among these was an organism which resembled *B. salmonicida* in pigment production.

#### Variability of *B. salmonicida*

##### Biological characters

The Furunculosis Committee (1933, 1935) found that *B. salmonicida* showed great uniformity in its biological characters and that variability was negligible. However, Duff (1937) found that by culturing *B. salmonicida* in special media (phenol broth and lithium chloride broth) variants arose, with distinct morphological and colony characters. This aspect of the study of *B. salmonicida* is dealt with separately in the next section under "Dissociation". The conclusions of the Furunculosis Committee (1933, 1935) with respect to biological uniformity in *B.*

Table 6.--Number of trout sera possessing specific agglutinins to dilutions shown <sup>1/</sup>

Serum dilutions	Total sera tested	Dilutions						No agglutinins
		1:10	1:20	1:40	1:80	1:160	1:320	
Vaccinated trout	37	2	2	9	8	7	1	8
Control trout	40	1	4	2	2			31

<sup>1/</sup> Duff, 1942.

Table 7.--Reciprocal relations of the R, S and G variants of B. salmonicida

Absorption antiserum	Cells	Cell suspension for titration		Dilutions of absorbed serum			
				1:40	1:80	1:160	1:320
<u>Group I (R = S + n)</u>							
R	S	R		a	a	a	a
S	R	S		-	-	-	-
R	G	R		a	a	a	a
G	R	G		a	a	a	-
S	G	S		a	a	a	a
G	S	G		a	a	a	-
R	R	R		-	-	-	-
S	S	S		-	-	-	-
G	G	G		-	-	-	-
<u>Group II (S<sub>1</sub> = R<sub>1</sub> + n<sub>1</sub>)</u>							
R	S	R		-	-	-	-
S	R	S		a	a	a	a
R	G	R		a	a	a	a
G	R	G		a	a	a	a
S	G	S		a	a	a	a
G	S	G		a	a	a	a
R	R	R		-	-	-	-
S	S	S		-	-	-	-
G	G	G		-	-	-	-

Modified from Duff (1939).

salmonicida are based on findings with media generally used in isolation and cultivation of that organism (agar, gelatin, milk, etc.). From a study of 120 strains of the organism they found general uniformity except for variations in: (1) gas production from glucose and mannite; (2) rapidity of liquefaction of gelatin and coagulated serum; and (3) rate and degree of pigment production.

Stevens and Keil (1931) isolated an atypical organism during an epidemic of furunculosis which they claimed later reverted to the typical B. salmonicida. That this was a true variant of B. salmonicida was disputed by Davis in a discussion of their paper during the annual meeting of the American Fisheries Society (1931). He did not doubt that they were dealing with true furunculosis, but he did feel that at the beginning of the epizootic, the symptoms did not fit the disease, and that the organism they isolated at that time did not represent a variant but a secondary infection. In spite of Davis' objections, it might be wise to relate the findings of these workers.

The organism of Stevens and Keil (1931) was obtained in pure culture from heart-blood and liver. It measured from 5 to 8  $\mu$  in length and from 0.8 to 1.1  $\mu$  in width. The ends were rounded and there was a tendency in many to taper slightly towards both ends. It was non-motile and Gram-negative. In the exact center of many of the organisms was a very pronounced unstained oval area that had every appearance of being a spore, and was so regarded by a number of observers who examined direct smears from heart blood and fluid of unbroken muscular lesions. Such organisms survived incubation at 37° C. but were killed at 57° C. In older cultures and upon transplanting the morphological appearances were changed and many shorter, typical furunculosis bacteria were present together with longer forms showing pair formation and bipolar staining (often noted in typical B. salmonicida). In all later transplants the organism had apparently reverted to forms usually encountered in investigations of furunculosis.

#### Dissociation--the formation of S, R, and G colonies

Duff (1937) has been the sole worker to date on this aspect of the study of B. salmonicida. He used a strain of B. salmonicida possessing the characters of a recently isolated sample. He subcultured the organism every 48 hours at temperatures between 19° C. and 21° C. in phenol broth and lithium chloride.

#### Dissociation in Phenol Broth Series (Duff 1937)

Duff (1937) found that three abnormal colony types arose out of the passage of the organism through phenol broth. From two serial tubes when plated on nutrient agar came giant colonies (5 to 8 mm. in diameter in 96 hours) in place of normal colonies (about 2 mm.). Typical brown

pigment was produced from these colonies. From a third tube of the series, in addition to the giant colonies came colonies with highly convex centers each surrounded by an annulus of thin, flat, transparent growth with entire edge. Platings from a fourth tube yielded again the colony types described for tube 3, and in addition numerous colonies, approximately 1.5 mm. in diameter, with a roughened surface. Examination with a hand lens showed that the roughness was due to the presence of large numbers of secondary papillae on and below the surface of each colony.

There were thus three abnormal colony types arising out of the passage of the organism through phenol broth--giant, annular, and papillated colonies. Further platings on nutrient agar were made from the giant colonies, from the central body and annulus of the annulated colonies, and from the papillae of the papillated colonies. Saline suspensions of the giant colony samples spread on nutrient agar plates showed an immediate reversion to the normal stock-colony type. Similar results were obtained with transplants of the annulated colonies.

In contrast to the complete reversion of these variants, platings from papillae showed colonies of the original type (stock culture type), and in addition about equal numbers of a new colony form, together with a number of colonies of an intermediate type. Selected colonies of the two distinct types when replated gave rise only to colonies of the same description as that of the parent colony, whereas platings from the intermediate type of colony always showed a further breaking up into two major and the intermediate colony types. These two major colony types were quite distinct. One was opaque, strongly convex, and cream-colored. The other was translucent, only slightly convex, and showed a slight but definite bluish-green color by transmitted light. The cream-opaque type proved nonpathogenic, the green-translucent, pathogenic.

#### Summary of *B. salmonicida* Dissociation in Phenol Broth Series

From Phenol Broth came--

- |                          |                                       |
|--------------------------|---------------------------------------|
| (1) Giant colonies.      | These colonies arose on nutrient agar |
| (2) Annulated colonies.  | after up to four subcultures in       |
| (3) Papillated colonies. | phenol broth.                         |

Giant and annulated colony types when spread on nutrient agar plates showed immediate reversion to the normal stock-colony type.

Platings from the papillae of the papillated-colony type showed colonies of the stock type, plus a new colony form and colonies of an intermediate type.

Selected colonies of the two distinct types when replated gave rise only to colonies of the same description as that of the parent colony.

Platings from the intermediate colony type showed a further breaking up into:

- (1) An opaque, strongly convex, cream-colored type of colony. This type was nonpathogenic, and was referred to as a "rough" type ("R"), (see below).
- (2) A translucent, only slightly convex, bluish-green type of colony. This type was pathogenic, and was referred to as a "smooth" type ("S"), (see below).

#### Dissociation in Lithium Chloride Broth Series (Duff 1937)

From lithium chloride the same stable variants arose, although the time required for their production was much longer than in phenol broth. The cream-colored opaque or "R" type of colony, as before, was nonpathogenic and the greenish-translucent or "S" was pathogenic. The cream-opaque or nonpathogenic type was found to be more stable on prolonged culture and produced flocculent growth in broth and friable colonies on agar. The greenish-translucent or pathogenic type produced on inoculation the typical lesions of the disease, was less stable on prolonged culture, and gave butyrous colonies on agar. These cultural types of E. salmonicida thus show respectively the major characteristics of the two classes of dissociant forms, rough (R) and smooth (S) first described by Arkwright (1921) for members of the colon-typhoid-dysentery group and since recorded by others for many species of micro-organisms.

#### Appearance of G-Type Cultures (Duff 1937)

During the period of dissociation of R and S type organisms in lithium-chloride broth tubes, another colony form appeared on one or two occasions. The colonies were very small (0.2 to 0.3 mm.), transparent, and perfectly circular, and only became visible after 72 hours' incubation. Slide preparations from these colonies showed a very small, Gram-negative, coccoid micro-organism (average 0.3 by 0.5  $\mu$ ), appearing in clumps in a manner similar to the arrangement of the larger E. salmonicida. Colonies of this third culture type were picked and inoculated on agar slants for future investigation.

After observing over twenty series in lithium chloride, Duff (1937) noted a sudden auto-sterilization in the tubes. Drops from one tube spread upon agar showed no visible growth even after 10 days' incubation. Aware of the work of Hadley et al. (1931), he suspected that a G-type organism might be present, and in order to demonstrate its existence, he decided to apply the Hauduroy technique to the contents of the tube.

This technique consisted in placing about 10 drops of broth from a Pasteur pipette on the surface of a primary agar plate (one spread direct from a "sterile" lithium-chloride tube, showing no growth after incubation). Just before drawing up the "sterile" broth into the pipette, the tip of the latter was bent in a flame to a slight angle. This allowed the pipette, after delivery of the broth, to be used as a spreader to distribute the fluid over the surface of the agar. A portion of the fluid was then collected in the pipette, transferred to the surface of two fresh sterile agar plates and spread as before. After 72 hours' incubation at room temperature, providing no G-type colonies appeared, one of two secondary plates was selected and the process was repeated as required.

When growth appeared, agar slant cultures were made from the colonies. The growth on these appeared as a flat transparent film on moist slants but tending to form discrete colonies on dried agar. Subcultures to broth after a few transfers on agar gave rise to uniform clouding in 72 hours.

Goldfish were inoculated intraperitoneally with this organism. No micro-organisms were recovered from these fish and no lesions developed.

Reference may now be made to the small colonies which appeared on agar plates of lithium-chloride broth series on one or two occasions during the phase dissociation of the parent culture. As previously recorded these had been transferred to agar for future examination. Replating showed that they were identical in all respects with the small colonies just described as having been recovered by special methods from apparently sterile lithium-chloride tubes. These earlier cultures were morphologically and biochemically identical with those obtained from "sterile" tubes.

Duff (1937) felt that the appearance of a minute colony form during dissociation, and later from apparently sterile tubes in lithium-chloride broth series of B. salmonicida corresponded most closely with the appearance under similar circumstances of G-type variants of the Shiga dysentery bacterium and of other species recorded by Hadley et al. (1931).

#### Recovery of Typical B. salmonicida from G-Variants (Duff 1937)

Of a number of stock G-strains maintained on nutrient agar, three reverted spontaneously to the R form after 12 months. Cultures of the R form, having exactly the same biochemical characteristics as the reverted G-strains, had previously been further changed to the pathogenic S form thus making a similar procedure unnecessary with these strains. There is, therefore, evidence of the existence of true G forms in B. salmonicida.

## Serological Relationships of S, R, and G Phases (Duff 1939)

Following his work on the formation of S, R and G colonies of B. salmonicida, Duff (1939) studied the serological relationships of these phases.

The technique followed with all sera was that of absorption with a low multiple of the minimal absorbing dose, followed by titration of the absorbed serum. Minimal absorbing doses for S and G cultures were always between 1:10 and 1:5, whereas the value for R cultures was never less than 1:2. Absorptions were carried out for 4 hours at 37° C. followed by ice-box storage overnight.

Considering first the R and S interconnections, five strains (CL6, CLII, SP5, ER2) were found to possess the same antigenic relationship between R and S phase cultures. In these strains the R cells of any given strain possessed an antigen or antigens not present in the S cells of the same strain. The antigenic situation may be represented in symbols as  $R = S + n$ . For convenience B. salmonicida strains so constituted were termed Group I strains. This relationship was not found to hold for certain other strains. Three strains (CL3, CL4, SP9) (Group II) displayed a different antigenic picture, in which the S cells possessed an antigen or antigens not present in the corresponding R cells, or,  $S_1 = R_1 + n_1$ .

### Typing Additional Strains (Duff 1939)

It proved impracticable to extend full reciprocal analysis to further individual strains of B. salmonicida. At the same time it was of definite interest to know whether other strains would all fall into one or other of the serological groups (in respect to the R and S relations). Therefore a tentative method for testing new strains was devised by Duff (1939). It will be seen that absorption, using homologous S cells, of an antiserum made against the R cells of any one strain from Group I yields what may be termed a "monovalent n" antiserum. A serum of this type was prepared by absorbing an antiserum made against the R cells of strain CL6 (Group I) with S phase of the same strain. The absorption was carried out twice (table 7).

Table 8 records the agglutinability of all strains under the influence of CL6 monovalent  $n$  antiserum. The results do not necessarily prove the complete identity of the CL6  $n$  antigen of the remaining Group I strains or with the  $n_1$  antigen of the three Group II strains. They do demonstrate however, that this antigen is common to R cells of all Group I strains and to S cells of all Group II strains.

In addition eleven other strains were tested in the same manner with similar results. Nine fell into Group I and two into Group II.

Table 8.—Agglutination of R and S phases of strains of Groups I and II by CL6 monovalents "n" serum<sup>1/</sup>

Phase	Group I					Group II		
	CL6	CLII	SP5	SPL3	ER2	CL3	CL4	SP9
R	a	a	a	a	a	-	-	-
S	-	?	-	-	-	a	a	a

<sup>1/</sup> Agglutination was at 37° C. for 4 hours. Results were read after overnight ice-box storage.

#### Antigenic Relationship of the G Variant to R and S Variants

Duff (1939) felt that the G phase cell might be composed in part of an individual G antigen or antigen complex, and in part of an antigen or antigens common to itself and to R and S cells of the same strain. He made no claim that all strains of B. salmonicida would necessarily fall into one of two serological groups in respect to the location of the n antigen in the R or S phase of the strain under consideration. Nevertheless, it is significant that 19 strains selected at random did so.

He also found that pathogenicity was not correlated with the antigenic picture elicited by the absorption methods used in his experiments.

#### BACTERIOPHAGE. IN RELATION TO CAUSATIVE ORGANISM

##### In Water

Christison and associates, who carried out a thorough study on B. salmonicida bacteriophage in 1938, collected water in a sterile stoppered bottle and added 200 cc. to 100 cc. triple-strength broth in each of five large flasks, carefully avoiding any contamination. One liter of water was used therefore in each case. Twelve strains of B. salmonicida isolated from cases of furunculosis in salmon and trout were chosen at random from a large selection of stock strains. Twenty-four hours' agar slope cultures of these were emulsified in broth and the emulsions were added to the broth-water mixtures in the flasks, three different strains being added to each flask. Since only twelve strains were employed, the fifth flask was inoculated with three of the strains already used, but in a different combination. In a number of cases the fifth flask was omitted, 1000 cc. of water being distributed in equal quantities among four flasks, in each of which 125 cc. of triple-strength broth had been placed.

The mixtures in the flasks were incubated at 20° C. for 24 hours. Then equal quantities of culture from each flask were thoroughly mixed together in a sterile flask and filtered through a Chamberland L5 filter. The filtrate was kept at room temperature for 48 hours to test its sterility and stored in an ice chest in glass ampoules or stoppered test tubes until required.

#### Technique in isolation and demonstration

Christison and associates (1938) then made stroke inoculations of the test organisms on agar plates with dense broth emulsions of 24 hours' agar slope cultures as the inoculum. After drying in the incubator at 37° C., drops of the filtrates were placed on the strokes and the plates were incubated at 25° C. for 48 hours. The results were then read, the presence of phage being indicated by the partial or complete absence of growth on the areas on which the drops had been placed. Four filtrates and four strains were tested on one agar plate.

A number of strains of B. salmonicida were used for the isolation of bacteriophage owing to the possibility of there being a number of types of phage. In such a case one or more strains of the organism might be resistant to one particular type of phage and thus prevent its development and recognition. The use of twelve strains, chosen at random, reduced this possibility to a minimum and rendered the isolation of phage from any particular specimen of water more likely.

The cultures of the strains to be used were also tested for the presence of phage, by growing them in broth in the same combinations and under exactly the same conditions as in examining river waters, and testing each against the combined filtrates. While the results indicated that weak phages were possibly present in some of the strains first used, it was very evident that the powerful phages obtained when river waters were added could not be accounted for by the enrichment of phage types already present in the cultures of the test strains.

#### Occurrence

In 1932 a bacteriophage for B. salmonicida was found in two separate cultures of the organism isolated from sea trout which had died of furunculosis in two Scottish rivers (Furunculosis Committee 1933). Todd (1933) also demonstrated a phage active towards B. salmonicida in the waters of certain English rivers. The phage has since proved to be widespread in natural waters throughout Great Britain (Christison et al 1938).

Various questions have arisen from the discovery of this lytic principle for B. salmonicida in natural waters. Todd (1933) thought that the presence of this phage in a river might be an indication of the presence of furunculosis of fish in that river. However, the results

of extended studies have not supported this idea. Christison and associates (1938) found that in twenty rivers in which phage was detected, there was no known furunculosis and in five instances no phage was detected where the disease had occurred. This phage was present and furunculosis had occurred, however, in fourteen cases. It has been shown to be absent from water in which an epizootic was in progress (Furunculosis Committee 1935). The lytic principle has been demonstrated in winter when the infection was in abeyance as well as in summer when the disease was prevalent; it has also been found in city sewage though absent from city water supply (Christison et al. 1938). The most recent work on this phage shows that it is impossible to correlate its presence or absence with the presence or absence of the disease or with the distribution of the infection. An explanation for the occurrence of this phage where no disease was known, may reside in the resistance of the phage. This may explain its widespread dispersion by water birds.

### In the Tissues of Salmon and Trout

#### Technique in isolation and demonstration

Christison and associates (1938) removed the gut and other organs (liver, heart, kidneys, and spleen) mincing them up separately in sterile dishes (the gut in one, the other organs in another). The two lots of viscera were then placed in separate flasks, each containing 300 to 400 cc. of broth. To each flask was then added a mixture of broth emulsions of 24 hours' agar cultures of twelve strains of B. salmonicida used in the isolation of phage from river waters. The resulting mixtures were incubated at room temperature for 24 hours. The cultures were then filtered separately through filter paper impregnated with kieselguhr to remove gross particles and then through Chamberland L5 filters. After being tested for sterility, the filtrates were stored in sealed ampoules or test tubes in an ice chest until required for use.

Each filtrate was tested against each of twelve strains of B. salmonicida, in the usual manner, to determine the presence of bacteriophage.

#### Occurrence

In human diseases such as cholera and dysentery, very active phages for the causal organisms have been obtained from the dejecta of infected persons, particularly in the convalescent stage of the disease. It was thought that this might occur in furunculosis, and Christison and associates (1938) examined a number of fish with this in mind.

In a number of cases active phages were isolated. It is of interest to note that the organs of some fish which had not died of furunculosis yielded phage, while the organs of fish which had died of the disease did not always contain phage, as far as could be ascertained by the technique used. Phage plaques were found in the primary cultures

of E. salmonicida from the heart blood of a fish that had died from furunculosis, indicating that the phage may occur in the blood stream. Besides occurring in various organs, phage was present on growths from lesions. Plaques were evident as soon as growth was visible before the characteristic brown pigment developed.

The occurrence of phage in primary cultures from fish dying from furunculosis could not be well correlated with any particular phase of an epizootic. Thus, in the case of a single furunculous fish taken in spring from a Scottish river, an exceedingly active phage was observed in the primary cultures of E. salmonicida; there had been no previous cases reported from this river in that year, and none occurred for some time afterwards. When phage was regularly observed to occur in primary cultures during the course of an epizootic, it appeared frequently in those made from fish in which the course of the infection had been unusually rapid and severe. Thus, in one case, a fish appeared to be in good condition as regards external appearances, but the viscera were so liquefied that the individual organs could hardly be distinguished.

#### Specificity

The specificity of E. salmonicida phage was first studied by Blake (Furunculosis Committee 1935), who tested it on strains of Escherichia coli, Shigella dysenteriae, water Vibrios, Pseudomonas fluorescens, and several strains of Achromobacter. It was found to be active towards E. coli and S. dysenteriae though less markedly than towards E. salmonicida.

Christison and associates (1938) tested the phage against a large number of organisms and they all were, with one exception, unaffected by the phage. This exception, a Gram-negative bacillus, was very susceptible to phage action. Indole was produced by this organism, nitrates were not reduced, and no pigment was formed.

E. salmonicida phage was tested against the following organisms and in no case was any evidence of phage action obtained: Micrococcus pyogenes var. aureus (two strains), E. coli (eleven strains), S. dysenteriae Shiga, Y and Sonne, Alcaligenes faecalis, Proteus morgani, Vibrio comma, Salmonella typhosa, S. paratyphi, S. suispestifer, S. typhimurium, S. enteritidis, S. pullorum, Pseudomonas aeruginosa, Serratia marcescens, and P. fluorescens. Besides these, seventy-one organisms isolated from water or from fish were unaffected by the phage (with one exception, given above). None of the prevalent bacteria in sewage were found to be carrying E. salmonicida phage.

## Types

### Technique in isolation and demonstration

The method used by Christison and associates (1938) for investigating different types of phage consisted of plating decimal dilutions of phage with a culture of a sensitive strain and incubating at 25° C. Discrete, well-separated plaques were "picked off" after 24 to 48 hours' incubation, placed in tubes of broth, which were inoculated with the same strain of B. salmonicida and filtered after 24 hours' incubation. After several strains of phage had been purified by repeating the process of plating, picking off discrete plaques, etc. several times, the final filtrate obtained was regarded as being approximately a pure type.

Resistant strains of the original sensitive culture of B. salmonicida were obtained to each of these final filtrates by inoculating 0.1 cc. dilutions of each filtrate with a sensitive culture and incubating until growth occurred. The sensitive strain of B. salmonicida used was "U.S.A. 5", a strain which was acted on by every phage preparation obtained, indicating that it probably was not resistant to the types contained in these filtrates. It had the further advantages that resistant strains were developed fairly easily from it, usually within 4 to 7 days; it also did not produce a diffusible pigment, which facilitated the reading of results.

Each of the final purified phage filtrates was tested individually against every resistant strain so obtained and the relationships between them were determined. Thus, if two phage preparations acted in the same way towards their respective resistant strains, they were considered identical, and if they had been purified sufficiently, it was assumed that they contained the same single type of bacteriophage until the contrary was proved. On the other hand, if they acted differently towards the respective resistant strains, they were regarded as different types of bacteriophage.

### Occurrence

Christison and associates (1938) found that it proved very difficult to isolate pure types of the bacteriophage, though a considerable amount of time was spent in picking off well-isolated plaques from successive generations of the same phages. Enough evidence was procured, however, to show that there were at least three different types in the specimens collected, and possibly more. One type was fairly readily separated, but the others showed some degree of overlapping.

### Origin

The origin of this phage is obscure. The propagation and presence of this phage in water in which furunculosis of fish is absent and from which B. salmonicida cannot be demonstrated is difficult to explain.

Christison and associates (1938) found that no prevalent bacteria derived from fish or water have proved to be susceptible to this phage or responsible for its propagation. On the contrary, however, Blake (1935) found Escherichia coli and Shigella dysenteriae susceptible to the phage though less markedly so than B. salmonicida. Since relatively little work has been done on this subject, one might feel reluctant to overlook the earlier conclusions of Blake (1935) for those of Christison and associates (1938).

Christison and associates (1938) considered whether a phage, originally associated with some water organism, under the conditions of experiments might become rapidly adapted to B. salmonicida and lose its activity towards its original bacterial host. However, they pointed out that while adaptation of a bacteriophage from one species of organism to another has been claimed (d'Herelle 1926), this has only taken place gradually, and it is difficult to postulate the rapid adaptation that would explain these findings. Christison and others (1938) also pointed out that the demonstration of this phage involved the bringing of water into contact with the laboratory cultures of B. salmonicida. Such cultures by themselves were proved to be free from phage and did not yield it when grown in broth under the same conditions. Therefore these results might even raise the question whether the phage originated de novo during the experiment. However, water which had been heated to 100° C. (to destroy any phage present in it) and then tested, did not lead to the demonstration of B. salmonicida phage.

The observations on B. salmonicida phage are of considerable interest but as Christison and others (1938) point out, no definite conclusions can be drawn apart from the fact that a phage active towards B. salmonicida and propagating with this organism in laboratory cultures is very widely distributed in natural waters, and that its presence in water cannot be necessarily correlated with the presence of furunculosis.

#### PATHOLOGY

The term "furunculosis" with reference to fish is a misnomer, for the lesions of this disease are not analogous to furuncles as they occur in the human subject. The so-called furuncles of fish are areas of necrosis in which there is no outstanding leucocytic infiltration. The pus-like discharge from these areas is not true pus, but consists of liquefied necrotic tissue showing microscopically muscle fibres, blood cells, and bacteria. However, the term "furunculosis" with reference to fish should stand, as time has given it priority.

The symptoms of the disease are not always obvious and those lesions present may be masked with fungus growth (Plehn 1911). Davis (1946) found in the case of fingerling trout that usually the only evidence of a lesion from the exterior is an irregular dark blotch just beneath the skin on one side of the body. This blotch ordinarily is

situated between the dorsal and pelvic fins and in young trout is probably the most characteristic symptom of the disease. Plehn (1911) states that fish may become a general dull color, and on occasion even blackish. Lethargy and quickening of breathing may also be present.

Furunculosis is properly described as a general infection in which focal lesions may or may not occur, according to the rapidity of its progress (Furunculosis Committee 1930). David (1926) claimed there were six forms of the disease. The Furunculosis Committee (1935) concluded that there were two main types of the disease: One which runs a rapid course, death occurring with no external signs, and termed "acute"; the other, termed "subacute," is slower in its progress, focal lesions developing in the muscles of various parts of the body. This is a better classification. Numerous variations from these two extremes do occur, including the carrier state (see below) which may last for a considerable period of time. In general, the infection may last from a few days to a few weeks or many months (Plehn 1911).

Williamson (1928) and Davis (1946) have emphasized the septicemic nature of the disease, bacteria being carried to all parts of the body in the blood stream. Often there is also focal destruction of the dermis with disintegration of capillary vessels. The underlying muscle is also involved in the destructive process, which extends into the connective tissue between muscle bundles. As described by Arkwright (1912) there may be multiple abscess-like cavities under the skin, sometimes of large extent containing an opaque reddish liquid. These cavities may be closed or open on the surface through small sinuses, and some may even penetrate into the body cavity (Furunculosis Committee 1930). Plehn (1911) has noted ulcers of the size and shape of a needle. Arkwright (1912) found these subcutaneous and muscular lesions most frequently along the lateral line, but Davis (1946) states that they ordinarily develop in the dorsal muscles, and sometimes have a marked tendency to form at or near the base of the dorsal or pelvic fins.

The kidney is frequently affected, and this organ may become necrotic and semifluid (Furunculosis Committee 1930). Plehn (1911) found that bacteria collected in the kidney in great numbers, especially in the glomeruli where they could be seen on sectioning.

The spleen may also be destroyed in the same way (Furunculosis Committee 1930, Duncan 1932). It is commonly enlarged and has a bright cherry-red color (Davis 1946). If the spleen is crushed underneath a cover slip (Davis 1946) white clumps of bacteria can usually be seen, even where there are no other indications of disease. They may also be seen on sectioning.

The liver is lighter in color than normally and has a tendency to become fatty. Minute hemorrhages are often seen and in microscopic preparations bacteria are found in and around blood vessels the walls of which have been damaged. Davis (1946) states that sectioning and staining

are almost a necessity before organisms can be seen in the liver. Plehn (1911) found that bacterial clumps were not as plentiful in the liver as in the kidney. She also found that the stellate cells of v. Kupffer were actively phagocytic.

There may be marked congestion of blood vessels of the abdominal cavity (Davis 1946) accompanied by petechial hemorrhages in the peritoneum, especially in the region of the swim bladder (Plehn 1911). The lining of the intestine and the pyloric region of the stomach may be inflamed, and there is often a discharge of blood and mucus from the vent. Plehn (1911) found that the mucosa of the stomach could be torn away from the submucosa with great ease. Congestion of the intestine is also common (Bouville 1907).

Congestion of the gills may also accompany any of the above symptoms (Davis 1946).

Table 9 shows the frequency of lesions found by Duff and Stewart (1933) during an epizootic in British Columbia. Note that in all cases there were muscle lesions and necrosis of the kidney. The Furunculosis Committee (1930) points out that the distribution of lesions is extremely variable from specimen to specimen. However, in diseased fish, the organism is most frequently demonstrated in heart blood and kidney. The Furunculosis Committee (1933) found that E. salmonicida was recovered less frequently from the intestine than from the kidney or blood.

As already indicated there is practically no leucocytic infiltration with furunculosis. Blake (Furunculosis Committee 1935) in a special study of this aspect of the disease found that films of blood of infected fish showed the presence of free bacteria in considerable numbers with relatively little phagocytosis. The first stages of an inflammatory reaction appeared to take place around the site of inoculation, especially if this was intraperitoneal. Vessels became dilated, plasma escaping into the surrounding tissues, but there was no definite migration of leucocytes and those present were seen to be degenerate. A certain amount of hemolysis occurred at an early stage of blood infection, and the progress of this was readily demonstrated by the observation of free nuclei of erythrocytes in blood films. A pronounced effect on the capillary blood vessels was manifested by the considerable degree of edema and the frequent occurrence of petechial hemorrhages.

Although in furunculosis engulfment of the causative organism by wandering mesodermal cells is not an outstanding feature, this is not true with all general infections of fish. Vascular reactions were found by Blake (1935) to be much more marked in cases of infection by vibrios, Achromobacter types, and Pseudomonas fluorescens than by E. salmonicida. Phagocytosis was very active by mononuclear cells (probably endothelial in origin), even more than by polymorphs. When

goldfish were inoculated subcutaneously with a pathogenic vibrio, progressive congestion of vessels around the site of inoculation was observed and films of subcutaneous tissue showed that migration of leucocytes had taken place and that definite phagocytosis was occurring.

Table 9.--Features of retaining-pond disease, British Columbia<sup>1/</sup>

Pathological feature (naked-eye observation).	Frequency of occurrence.
Muscle--large (0.5 cm. to 10 cm.) closed lesion, containing fluid or semifluid reddish "pus", subcutaneous or deep.	27
Muscle--large lesions open to the exterior but not located at the base of the pectoral or pelvic fins.	Number of fish showing one or more types of muscle lesions--66. 10
Muscle--small hemorrhagic dots or streaks in,	41
Kidney--liquefactive necrosis of,	66
Spleen--liquefactive necrosis of,	57
Liver--hemorrhage or lesion of,	25
Intestine--congestion of any portion of,	61
Intestine--marked congestion of posterior part with or without congestion of other portions,	43
General visceral congestion or peritonitis,	13
Free body fluid in body cavity,	12
Vent--exudate of blood and mucus from,	50
Pelvic fins--subcutaneous hemorrhage at base of,	32
Pelvic fins--open lesions at base of,	Total lesions at base of pelvis-- 40
Pectoral fins--subcutaneous hemorrhage at base of,	19
Pectoral fins--open lesions at base of,	Total lesions at base of pectorals --34. 15
Anal fin--subcutaneous hemorrhage at base of,	22

<sup>1/</sup> After Duff and Stewart (1933).

No active toxin or filterable agent has been demonstrated in furunculosis (Furunculosis Committee 1935). Filtrates of a spleen of an infected fish were injected into two fish but no signs of disease developed even after 1 month. Two control fish inoculated with unfiltered emulsion each died in 5 days with B. salmonicida being recovered from

both. No toxin could be shown in cultures of the organism. While the possibility of toxin production in the body of the host could not be excluded, the Furunculosis Committee (1935) felt that the pathogenic action of E. salmonicida was largely due to its abundant growth in blood and tissues thus interfering with the blood supply resulting in necrosis and liquefaction of tissues. The organism of furunculosis also exhibits a marked proteolytic action.

Field and associates (1944) have shown that there is a rapid decline in blood sugar in an acute infection with E. salmonicida. They found that there was a drop from the normal of approximately 100 mg. percent to the abnormally low level of 5.8 to 12.3 mg. percent. Their findings suggest that when the inoculum is large the rapidly multiplying organism utilizes blood sugar as a convenient source of energy, inducing a hypoglycemic shock which may be fatal within 3 to 5 days. In a more chronic infection, the non-protein nitrogen content increased to phenomenal amounts, values of 600 to 700 mg. percent being not uncommon, while analysis of control samples gave results of approximately 30 mg. percent. For the most part they found that the increased nitrogen consisted of amino acid nitrogen, values of 400 to 500 mg. percent being recorded for some fish. They also discovered variable increased quantities of urea, which in some cases accounted for a substantial fraction of the rise in nitrogen samples. However, Field and associates (1944) stressed that increase in urea values may only reflect a progressive uremia concurrent with or as a result of kidney degeneration. On the other hand, the rise in amino acids as well as creatine is probably a result of intensive degeneration of muscle tissue. It is conceivable that creatine can be utilized by the bacteria as a ready source of energy (Dubos and Miller 1937).

Hemoglobin, red blood cell count, total plasma protein, albumin and globulin in inoculated fish were found by Field and associates (1944) to be essentially the same as the control animals. An unusual amount of oil globules was always observed on the surface of accumulated visceral fluid of autopsied animals. Likewise, oil globules, never observed in control animals, were present in blood taken from all diseased fish.

Agglutinins are formed in the sera of fish (carp and several species of trout) and turtles (painted and snapping turtles) infected with E. salmonicida (Smith 1940, Gee and Smith 1941, Duff 1942). The highest titer recorded is 1:2,560, observed from the sera of a turtle (Gee and Smith 1941). The highest titer recorded for trout is 1:640 (one rainbow and one brown trout held at 10° C. (50° F.)) (Smith 1940). Cushing (1942) found that the antibody-titer of fish kept in a warm tank rose sooner than those kept in a cold one, indicating, that for fish at least, temperature influences the rate of antibody production.

## PATHOGENESIS

It is not the purpose here to deal with variations in susceptibilities or methods of spread of disease or any other factors which presuppose pathogenesis, as these will be dealt with in the next section. This section will serve as an introduction to the section that follows.

Besides being pathogenic to members of the Salmonidae and other fish in nature, B. salmonicida has been shown experimentally to be infective, by various routes, to a number of other fish and cold-blooded vertebrates. Williamson (1928) showed that infection could be set up in frogs, minnows, and goldfish (Carassius auratus). Intraperitoneal injection with a large inoculum of B. salmonicida was necessary to kill frogs, which suggests that it is unlikely that they would be infected in nature. She also succeeded in infecting a blenny by intraperitoneal injection of 1/10 of an agar slope of B. salmonicida. The organism of furunculosis cannot establish itself in the tissues of warm-blooded animals (Marsh 1902, Williamson 1928). Although susceptible by injection, eels do not readily contract the disease, as attempts to infect them by feeding contaminated food or contact with diseased fish have been unsuccessful (Furunculosis Committee 1933).

Results of experiment clearly show that healthy ova from infected parents are not invaded by the organism and that the organism is not transmitted from parent to progeny through either ova or sperm (Furunculosis Committee 1935).

It is concluded by the Furunculosis Committee (1935) that the bacterium may invade a new host from water by the gills, alimentary tract, or in some cases a surface injury, though it proved impossible to state definitely the common natural route.

### DETERMINING FACTORS

#### Susceptibility of Varicous Fish to the Disease in Nature

##### The Salmonidae

The disease in nature is limited to fish living in fresh water and it has been recorded mainly among members of the family Salmonidae (Furunculosis Committee 1930). As has been shown experimentally, other fish and cold-blooded animals can also be infected (see above).

#### Variation Among Species and Individuals

Table 10 summarizes the comparative susceptibilities of various members of the Salmonidae as suggested and found by a number of workers, with the least susceptible shown at the bottom of the table. Table 10 shows that there is general agreement amongst various workers that rainbow trout (Salmo gairdnerii) are least susceptible.

Blake and Clark (1931) found that when Salmo trutta (brown trout), Salvelinus fontinalis (brook trout), and S. gairdnerii (rainbow trout) were exposed to infection by contact with inoculated fish or by adding B. salmonicida to the water, S. trutta was most susceptible, S. gairdnerii was highly resistant, and S. fontinalis was intermediate in susceptibility. They exposed seven S. trutta, eight S. fontinalis, and eight S. gairdnerii to infection by contact with one trout inoculated with B. salmonicida. All seven brown trout became infected and died; one S. fontinalis died of acute furunculosis, and when the remainder which were apparently healthy were tested by kidney puncture, four proved to be carrying the infection and subsequently died, while three had resisted infection. None of the rainbow trout was infected as was revealed by kidney puncture.

In a second experiment carried out by Blake and Clark (1931) these species were exposed to infection by adding cultures of B. salmonicida to water. All seven S. trutta again succumbed to infection; of eight S. fontinalis seven became infected, but three of these did not die until after kidney puncture. Of nine S. gairdnerii, one harbored a latent infection and died after kidney puncture, while eight proved resistant.

According to Blake and Clark (1931) in many rivers in which S. trutta, S. truttae (sea trout), and S. salar salar (Atlantic salmon) exist side by side, dead of the last two species are found while the brown trout (S. trutta) appears to escape.

The Furunculosis Committee (1933) found that grilse (S. salar salar) and sea trout (S. truttae) were highly susceptible experimentally, and the proved mortality from furunculosis in the former was 86 percent and in the latter 100 percent.

From the results of experiments, the Furunculosis Committee (1935) showed that among a colony of brown trout (S. trutta) usually only one-third to one-half exposed to infection contracted the disease whereas adult sea trout (S. truttae) are much more susceptible, the mortality rate being over 80 percent in one instance. They also concluded that there is no essential difference in susceptibility between hatchery brown trout (S. trutta) and wild brown trout.

According to Belding (in Hayford 1921) landlocked salmon (S. salar sebago) are more, and rainbow trout (S. gairdnerii) less, susceptible than brook trout.

Other Salmonidae which have been found to be susceptible in nature are the Dolly Varden trout (Salvelinus malma spectabilis) (Duff and Stewart 1933) hybrid salmon (Oncorhynchus sp.) (Duff 1933) and lake trout (Cristivomer namaycush) (Marsh 1903).

Table 10.--Comparative susceptibilities of various Salmonidae

Emmerich and Weibel 1894 (after Blake and Clark 1931)		Belding, 1921 (in Hayford 1921).	Plehn 1924	Blake and Clark 1931.
				<u>Salmo salar salar</u> and <u>Salmo trutta</u> (sea trout, Europe)
<u>Salmo trutta</u>	<u>Salmo salar sebago</u>	<u>Salvelinus fontinalis</u>	<u>Salmo trutta</u>	
	<u>Salvelinus fontinalis</u>	<u>Salmo trutta</u>	<u>Salvelinus fontinalis</u>	
<u>Salmo gairdnerii</u>	<u>Salmo gairdnerii</u>	<u>Salmo gairdnerii</u>	<u>Salmo gairdnerii</u>	
Furunculosis Committee 1931.	Furunculosis Committee 1933.	Furunculosis Committee 1935.	Davis 1946.	
				<u>Salmo trutta</u> and <u>Salvelinus fontinalis</u> (particularly susceptible).
	<u>Salmo salar salar</u> (grilse) and <u>Salmo truttae</u> (highly susceptible experimentally).	<u>Salmo truttae</u>	<u>Salmo clarkii</u>	
<u>Salmo gairdnerii</u>		<u>Salmo trutta</u>	<u>Salmo gairdnerii</u>	

The Furunculosis Committee (1933) found that in experiments in which trout were exposed to infection by contact and by contaminated food or water, individual fish of the same species exhibited great variation in susceptibility. Some resisted infection completely, others developed the carrier state (see below) and ultimately died of active disease after a variable period. Still others developed the "subacute disease" with external furunculous lesions, and again others died rapidly of a general infection without localized lesions.

#### Influence of Age

That susceptibility increases with age, which has been found in Europe (Plehn 1924, Blake and Clark 1931), has not been the experience in the United States where fingerlings and yearlings frequently suffer

heavy losses (Davis 1946). Davis (1946) states that among mature trout the disease is usually rare except during and shortly after the spawning season when the fish are particularly susceptible to infection. However, Plehn (1924) states that fry contract the disease rarely in Germany, and Blake and Clark (1931) in Great Britain found that of twenty-five brown trout (S. trutta) which had died of furunculosis in rivers and submitted to the laboratory for examination, all were at least 3 years old. Similar results have been found by Blake and Clark (1931) in the case of furunculosis in hatcheries, trout of 2 years of age and older being most frequently affected. Among thirty-seven infected brown trout from a hatchery examined by these workers, ten were 2-year-old and twenty-seven were 3 to 5 years. Laboratory experiments by Blake and Clark (1931) support the view that in Great Britain at least susceptibility increases with age. Table 11 shows their results.

Table 11.--Susceptibility of S. trutta at different ages. S. trutta were variously exposed to infection (B. salmonicida added to food and to water, water from infected fish, and contact with infected fish).

Age of fish.	Number exposed to infection	Number infected
Hatchery fry.	180	0
Hatchery yearlings.	24	5
Two-year-old.	44	38
Three-years, wild Loch Leven.	14	12

The conditions under which trout of different ages were exposed to infection were as far as possible the same, and the same strain of B. salmonicida was used throughout the series of experiments tabulated. In support of the findings of Blake and Clark (1931) in Great Britain, the Furunculosis Committee (1933) also found that finnock (S. truttae) showed a lesser susceptibility than adult sea trout.

#### Other fish

Surbeck (1909) reported furunculosis among grayling (Thymallus vulgaris) in Europe and Duff (1932) reported it among grayling (Thymallus signifer) in the Elk River, British Columbia. Duff and Stewart (1933) also found it in Rocky Mountain whitefish (Prosopium williamsoni) in British Columbia. Arkwright (1912) found it among chub and dace in the Wye River (Great Britain).

Other fish in which the disease has been found in nature are: tench (Tinca tinca), carp (Cyprinus carpio), catfish (Silurus glanis, Cambridge Natural History classification), pike (Esox lucius), and sculpins (Cottus spp.) (Furunculosis Committee 1930). Plehn (1911) has noted it in perch (Perca flavescens).

#### Recovery from the disease

There is only one record of a definite recovery from furunculosis without treatment. In a rainbow trout which Blake and Clark (1931) had inoculated, a furunculosis lesion was formed in a few days but soon subsided. Some months later the fish was found to be free from infection.

#### Sources of Infection and Methods of Spread of Disease

##### Spread of disease by water and food

Emmerich and Weibel (1894) and Plehn (1911) first showed that water would probably serve as a vehicle for the spread of furunculosis. Horne (1928) added three broth cultures to a sink of circulating water containing twelve gobies, stopping circulation each time for 6 hours to increase chances of infection. Five fish died between 15 and 26 days after the last exposure to infection.

Blake and Clark (1931) succeeded in bringing about the spread of disease by placing one infected trout in a tank with healthy fish. Of six healthy contacts the first died in 14 days and the fifth in 120 days. The other survived. The Furunculosis Committee (1935) showed that dead fish readily spread the infection, even at low temperatures.

Blake and Clark (1931) also showed that the spread of infection could be brought about when healthy fish were not in actual contact with diseased specimens. In one experiment a tank was divided into two compartments by a perforated double grid permitting free interchange of water but keeping the fish separate. On one side were placed two inoculated trout and on the other healthy fish. In less than a month five of six previously healthy trout died of furunculosis.

Clayton (1927) infected brown trout by giving them food contaminated with B. salmonicida.

##### Carriers

Early in the study of furunculosis the occurrence of carriers of B. salmonicida was evident. Arkwright (1912) cultured B. salmonicida from apparently healthy fish which had been bred in captivity. He suggested that these fish were in the incubation stage of the disease. Plehn (1909) and Mulsow (1913) pointed out that B. salmonicida may be

present in the intestine of outwardly healthy fish. Horne (1928) showed by laboratory experiments that "healthy" carriers were an important source of infection to healthy fish. Carriers harbor the organism mainly in the kidney (Furunculosis Committee 1933).

### Occurrence

Arkwright (1912) records that of twenty-three trout which were regarded as healthy controls for experimental work, four yielded cultures of B. salmonicida. All these came from a single hatchery. On examining eighteen live trout from the Kennet River, Horne (1928) found that three were harboring the specific organism. Williamson (1929) discovered seven carriers from a trout rearing station. Three were detected from a group of eighty brown trout and four from a group of nineteen rainbow trout.

In one district where furunculosis had been epizootic for 6 years, the Furunculosis Committee (1933) found two carriers.

The carrier rate in nature no doubt depends on the presence or absence of an epizootic and the trend of an individual epizootic. During the initial peak of an experimental epizootic, it has been shown to be as high as 34 percent (Furunculosis Committee 1935).

The evidence indicates that carriers are not confined to any age group or to any species of Salmonidae. Blake and Clark (1931) found them among 2- and 3-year-old brown trout (S. trutta). They have been found among rainbow trout (S. gairdnerii) and 2-year-old Atlantic salmon (S. salar salar). Probably any species of fish susceptible to the disease is a potential carrier.

After an experimental epizootic had subsided, the Furunculosis Committee (1935) made a most thorough examination for carriers among the remaining stock. Cultures were made from the heart blood and kidney in all cases and in most cases the contents of the lower intestine, spleen, and liver were cultured. Of 151 brown trout examined only 4 carriers were detected. In each case B. salmonicida was found in the kidney, but only one fish carried the organism in the liver. After this experimental epizootic had subsided, the carrier rate was 2.7 percent.

As already mentioned, it has been shown experimentally that at the initial peak of an epizootic the carrier rate is much higher—34 percent of the live fish netted for examination. The proportion diminished progressively and by the time cold weather had set in (December), no infected fish could be discovered in the surviving stock (Furunculosis Committee 1935).

## Significance and Nature

Horne (1928) was well aware of the importance of carriers when he stated that "carriers are in all probability a chronic source of infection to healthy fish in a river." He felt that a knowledge of carriers would be of great value, since it would throw light on such obscure points as the spread and persistence of disease. The difference in the carrier rate in healthy years and epizootic years would be valuable information. It might be possible to gauge the probable course of the disease in a given season and test the effect of such preventive measures as avoidance of stocking and the removal of ill-conditioned fish from a river (Horne 1928).

At first the Furunculosis Committee (1930) was undecided whether carriers of furunculosis were convalescent or paradoxical. In the former the animal has gone through an attack of the disease, and though recovered, still continued to harbor the specific organism in some part of its body. The paradoxical carrier is an animal harboring a pathogenic organism without having suffered any recognizable disease. This type of carrier apparently possesses a certain degree of tolerance towards the infection but later may succumb to an overt attack.

In support of Arkwright's (1912) original suggestion, Blake and Clark (1931) found that carriers which survived the infection studies proved to be of the incubation type, that is, the infection remained latent for a variable period but eventually the disease developed in fatal form. The work of Blake and Clark (1931) has since been thoroughly supported (Furunculosis Committee 1933, 1935), and it is now recognized that the true nature of furunculosis carriers is incubatory. In three cases, they noted that a flaring up of infection occurred for no apparent reason, trout dying of furunculosis after remaining alive for at least 4 months after exposure to B. salmonicida. In three other cases, they found that carriers did not recover after kidney puncture but died of general infection by B. salmonicida, indicating that the disease might remain latent until stimulated by some circumstances injurious to the host.

In order to demonstrate whether adverse conditions of a less violent nature would also lead to development of active disease in carriers, Blake and Clark (1931) performed a test in which five trout suspected of being carriers were subjected to a gradually increasing temperature (5° to 18° C.). This is comparable to a change from winter to summer temperature but taking place much more rapidly. Four of five trout died in 4 to 6 days, the survivor proving to be free from infection. The Furunculosis Committee (1933) also demonstrated the death of carriers following a rise in temperature. These facts are of special interest since in nature furunculosis is usually prevalent in summer, and it suggests that trout become infected in summer, harbor a latent infection during winter, and die of active disease when the temperature rises the following summer.

This assumption was borne out by studies of the Furunculosis Committee in 1935. In May 1934 a fresh batch of trout was added to a colony which had the previous year experienced an epizootic during experimental work. When the temperature rose to 50° F. (10° C.) during that month, furunculosis appeared in epizootic form. The presence of the disease in the added batch was excluded by a control observation. This result demonstrated directly how the infection may be maintained in latent form during winter to reappear the following season through the medium of carriers. Although in April, a sample from this colony did not reveal carriers some must have persisted.

#### Stocking in relation to the disease

In some trout fisheries in England, an epizootic has immediately followed the introduction of trout from a farm known to be infected, and in several instances such importation from a trout farm can be traced to rivers where the disease has occurred among salmon. In one infected area it was found that fry reared from locally obtained ova were fed on food supplied from a farm known to be heavily infected. In one case the disease was confined to that section of the river into which trout from an infected farm had just been introduced. Several epizootics were reported in widely separated districts in which fry from another farm had just been imported. In another river brown trout had been placed from a known infected farm, and a high mortality reported, although the disease was not observed in closely adjacent rivers in which trout from a farm believed to be clear of the disease had been introduced. The distribution of fish from farms in which furunculosis is present must involve, therefore, the risk of introducing new sources of infection among the fish population in natural waters (Furunculosis Committee 1930).

In Great Britain it has been shown that continuous introduction of the disease may be taking place through imported foreign trout. It has, in fact, been thought that the infection was originally introduced into Great Britain by infected fish from the Continent of Europe (Furunculosis Committee 1930).

#### Migratory Salmonidae as a source

It is difficult to conceive the propagation of furunculosis among salmon or other fish in the sea as it has been shown that the specific organism survives for only a short time in sea water (see above). Field observations and experiments (Furunculosis Committee 1930, 1933, 1935) have shown that active spread of furunculosis does not occur to any extent where the volume of water per single fish is great. Again, temperature conditions in the sea would generally be unfavorable to the development and spread of the disease. There is no proof of the existence of the disease among salmon caught by nets in sea water even in infected districts or of its occurrence in sea fish. Among salmon netted at the mouths of rivers and examined for B. salmonicida, no instance of

infection has been found. There is no evidence of smolts carrying the infection from infected waters on their migration to the sea. Large numbers of smolts from a river in Great Britain in which the disease had been exceedingly prevalent were examined with completely negative results.

If the disease did occur among salmon in the sea, it would be difficult to explain why fish of one river should be infected and not those of another, since salmon which finally enter different rivers are undoubtedly closely associated on approaching the coast. On the basis of a sea origin, the partial distribution of the disease among different rivers in Great Britain would be inexplicable, especially when an infected and uninfected river may be close to one another (Furunculosis Committee 1935). For instance, furunculosis was found present in the Forss, but not in the Halladale or Thurso; rivers which enter the sea a few miles on either side of the Forss. Furunculosis was present in the Donn, but nonexistent in the Ayr only a mile away. Were the disease generally present in the sea, such selective distribution in rivers would be highly unlikely.

In addition, were the infection introduced from the sea, one would expect to find the disease invariably showing at or near the mouth of each river, whereas in certain rivers in Great Britain furunculosis was first seen many miles from salt water (Furunculosis Committee 1935). Again, furunculosis has appeared in trout streams in which migratory fish, except eels, do not reach and in trout farms from which even eels are rigidly excluded.

The disease can be transmitted through a marine channel through sea trout. In Great Britain they are often in the habit of migrating during autumn from estuary to estuary. These wandering trout may conceivably visit an infected area and should an epizootic be in progress at that time, then one or more of these temporary visitors might also become infected and carry the bacterium with it to another estuary at no great distance. Of such transmission there is no direct evidence, although it might conceivably account for one or two outbreaks the origin of which is obscure (Furunculosis Committee 1935).

It has been shown by large-scale experiments (Furunculosis Committee 1930, 1933, 1935) how rapidly grilse and adult sea trout contract the disease and die from it when placed in water with infected brown trout. The infection often falls chiefly on salmon and trout in Great Britain which have left the sea only a short time previously. The Furunculosis Committee (1930) takes the stand that such fish on entering a river in which the disease exists become rapidly infected, due to environmental changes. They support the suggestion that salmon entering a river may contract the disease in 4 days to 2 weeks' time, become weakened, pass down the river to the estuary or even into the sea, reascend as far as the estuary, and die. Thus, fish passing into an infected river might well succumb to the disease while still carrying sea lice, but the committee feels that the occurrence of the infection in

migratory Salmonidae even at this early stage of their adult life in fresh water cannot be accepted as any indication of a marine source of the disease.

The Furunculosis Committee (1930) does not believe that salmon contract the infection in fresh water prior to smolt migration and continue to harbor it in a latent form during their life in the sea, developing active disease on return to fresh water. As noted above they have not found B. salmonicida in members of the Salmonidae netted at or near the mouths of rivers emptying into the sea. Their examination of large numbers of smolts (1339) from the Coquet proved negative. Although it is not impossible that a few smolts may carry the infection to the sea, it is improbable in view of the selective distribution of the disease (see above) that enough do so to be of serious epizootiological importance. In view of viability studies it is most certain that furunculosis does not spread in the sea.

#### Other sources

Although susceptible by inoculation, attempts to infect eels by contact and by feeding infected material have been unsuccessful. Other experiments have indicated that in nature eels do not readily contract the infection or spread it. It has been shown that B. salmonicida may be carried on the surface of ova.

#### Factors Favoring the Prevalence of the Disease

##### Water temperature and floods

The factors predisposing to epizootic prevalence of the disease constitute a question of utmost importance in its prophylaxis. In Great Britain the disease has generally proved seasonal in its incidence, outbreaks usually occur from the end of May to October, there being a relative quiescence during the winter and spring seasons (Furunculosis Committee 1930). In a hatchery outbreak recorded by Belding (1927), the disease appeared in winter but the mortality was slight. However, when the temperature rose above 55° F., deaths increased and when it reached 60° F. the infection became epizootic, spreading from pond to pond. Observations tend to show that warm dry weather favors the prevalence of disease, and that the occurrence of floods brings to an end outbreaks.

Further observations by the Furunculosis Committee in 1933 have indicated that water temperatures must be within the range of 55° F. to 66° F. before an epizootic of important dimensions is likely to take place among members of the Salmonidae in nature. Experimentation has further supported this view (see below). In one case it was found that until water temperature had reached over 55° F., in the beginning of June, no deaths from furunculosis occurred, but thereafter, until the night temperatures remained consistently below 55° F. in the latter half

of September, fish which had died of furunculosis were found regularly. Both before and after these periods the effects of the disease were not apparent. In another case the Committee observed that after a rise in temperature and a fall in water level an outbreak of furunculosis occurred. Thus during the months when water may easily reach a critical temperature, the sequence of events appears to be as follows: After a flood, fish ascend from the sea or from lower down in a river; they congregate in pools; the water level falls; they acquire the infection and rapidly succumb. This need not be invariably the case, since in other districts in Great Britain fish die of the disease, though not in such great numbers, where there is no fall in water level nor any great aggregation.

If given a suitable temperature in an area where an infection exists, and provided there is any aggregation of salmon, an outbreak is almost certain to take place, unless the water level is such that the fish continue their movement upstream, giving them the best possible conditions for maintaining their health (Furunculosis Committee 1933). Areas of greatest mortality are those where the fish are checked by an obstruction in the river, and where of necessity they must collect in considerable numbers relative to the size of the body of water. If, in such a situation, the fish are fresh-run the mortality is more serious. On the other hand, fish fresh-run from the sea is by no means a necessary condition for an outbreak, nor does resistance for any particular length of time in fresh water render them immune.

The Furunculosis Committee (1933) also undertook experimental work to determine the influence of temperature on the prevalence of the disease. Tanks with thermostatic controls were used, and running water was well aerated and mixed by compressed air. Infection was produced by contact with trout which had been inoculated intramuscularly with B. salmonicida cultures. The occurrence and development of disease was observed at different temperatures -- 5° C. (41° F.), 13° C. (55° F.), 15° C. (59° F.), 18° C. (64° F.), and 21° C. (70° F.).

Death from furunculosis resulted more rapidly at higher temperatures (up to 21° C.), but there was an apparent optimum temperature for the spread and development of the disease, the incidence being greater at about 15° C. than at 21° C. or at 5° C. Thus, both very low and unusually high temperatures may reduce the prevalence of the disease among fish exposed to infection under experimental conditions (Blake and Clark 1931, Furunculosis Committee 1933). These findings accord in part with observations in nature that a favorable temperature range for the spread of the disease is from 55° F. (13° C.) to 66° F. (19° C.).

Blake and Clark (1931) have suggested that it is possible that the susceptibility of trout to the disease actually increases with a rise in temperature up to a point where the temperature itself is definitely harmful to the fish. As the optimum temperature for growth of B. salmonicida is about 20° C., the falling off in incidence of infection

at 21° C. does not appear to be due to lessened activity of the organism itself. The Furunculosis Committee (1933) feels that it is doubtful whether the greater incidence of infection at about 15° C. under conditions of experiment can be exactly related to the optimum temperature for growth of B. salmonicida. They add that an important factor is the time an infected fish takes to die of the disease, which in turn is dependent on the height of the temperature. Thus, a fish dying rapidly probably contaminates its surroundings to a lesser degree than one in which the illness is prolonged. The optimum temperature for spread of the disease may be dependent therefore not on one factor but a combination of influences acting together. At very low temperatures, it is probable that the direct effect of temperature on the bacteria is a factor which lessens the chance of infection.

#### Water pollution

In Great Britain the majority of epizootics have taken place where pollution is either infinitesimal or nonexistent (Furunculosis Committee 1930). In the Tweed (Scotland), a heavily infected river in certain parts, the incidence of the disease was so slight that only by chance has it been identified. Only in the Lledr (England), an infected tributary of the Conway, and in some of the southern trout streams in which the disease has occurred, was pollution stated to be of any importance. In Great Britain, furunculosis has not been found to be restricted to any particular type of river but has occurred in chalk and peat water, in mountain streams almost free from pollution and in heavily polluted rivers (Blake and Clark 1931). Horne (1928) found no distinct relation between high bacterial content of water and the presence of the disease, and in his viability studies could not recover the organism from sewage after 48 hours.

Plehn (1924), on the other hand, found not only that B. salmonicida survived longer in polluted than in pure water, but also that the danger of infection increased according to the quantity of pollution by organic matter. According to her effluents from breweries, distilleries and dairies are most harmful and in them the disease takes its worst form. In support of Plehn's (1924) view, Duff (1940) showed that B. salmonicida survived 15 days in undiluted fresh sewage and at least 30 days in diluted autoclaved sewage.

It becomes evident at once that not only is the information regarding the relation of the prevalence of furunculosis to pollution contradictory but that the problem is also a complex one. It appears that the crux of the matter resides in the nature of the pollution. Sewage high in organic content might well favor the survival of B. salmonicida partly in itself and partly by enhancing growth of other flora, bacterial and otherwise, already present in fresh water, thus favoring the development of a severe epizootic. On the other hand, noxious inorganic matter (such as found in trade wastes) might well cut

short the survival time of B. salmonicida, thus being from the organism point of view an inhibitor of epizootic spread of disease. Again, organic or inorganic pollution could lower the resistance of fish in a river or stream to such a degree that almost regardless of its effect on the number of B. salmonicida present, an epizootic would develop. This seems to the author the wisest stand to take on the question of pollution in relation to furunculosis.

#### Lack of oxygen

Since in nature, deaths from furunculosis frequently take place among fish which are collected in pools where they may have insufficient oxygen, the Furunculosis Committee (1933) attempted to simulate this environmental condition in experiments. By keeping water temperature low, deprivation of oxygen per se could be tested.

Ten yearling brown trout, exposed to infection some months before, were kept in still water for several days. Two died in 3 days and two in 4 days, and one of each pair was found to have B. salmonicida in the kidney. The remaining six fish were in great distress on the sixth day and were killed; the kidneys of two yielded cultures of B. salmonicida. This result suggests that yearling trout which are harboring a latent infection in their kidneys are no more liable to die when the oxygen content of water is diminished than are healthy fish (Furunculosis Committee 1933).

Ten 2-year-old brown trout were exposed to infection by contact with an inoculated fish and survived for 28 days. The supply of water to the tank was then shut off, and in 24 hours two fish were dead. B. salmonicida was isolated from the heart, blood, and kidneys of both. In 48 hours the remaining eight fish died, and one of these proved to be infected. In this experiment with 2-year-old trout, the Furunculosis Committee (1933) concluded that fish in which the disease had reached the bacteremic stage were less able to withstand decrease in oxygen.

#### Other factors

The question arose to the Furunculosis Committee (1933) as to whether or not the spread and development of the disease is influenced by the hydrogen-ion concentration of water. Observations of the disease in various natural waters, including peaty waters and hard water streams, have not suggested that the type of water influences the development of the disease (Blake and Clark 1931). An attempt was made by experimental methods to ascertain whether or not water to which peat moss had been added in one case and chalk in the other affected the development of furunculosis in trout as compared with ordinary tap water. It was found that B. salmonicida did not retain its viability as long in peaty water (having a pH of 6.6) as in tap water and water containing chalk, but infection could be set up in trout kept in each type of water when culture was added to the tank.

Wolf (1936) found that cottonseed meal had a bad effect on the resistance of trout to furunculosis. He concluded that pure meat, and a diet consisting of one part meat, one part dry skim milk, one part fish meal, and one part salmon-egg meal were the diets most effective in producing disease resistance.

Course of infection set up by diseased fish  
with healthy contacts

After a colony of fish was brought into contact with the infection, the peak of disease occurred within four to nine days (Furunculosis Committee 1933, 1935). They found that the most susceptible individuals contracted the disease at this time and then died after varying intervals, as long as the temperature remained favorable, and a certain portion of fish served for a while as incubatory carriers.

### DIAGNOSIS

While in some cases a presumptive diagnosis of the disease might be made by observation of external appearances of dead or diseased fish, other conditions may be confused with furunculosis (see below), especially by persons who have no technical knowledge of the disease. Furthermore, it has been clearly shown how a fatal infection may occur without characteristic external lesions, and how apparently healthy fish may carry the organism. An accurate diagnosis can only be made by isolation and bacteriological examination of the specific organism. Identification by morphological, cultural, and biochemical reactions can be relied upon, and pathogenicity tests supply confirmatory evidence. The complement fixation test is also excellent for confirmation but is not necessary. According to the Furunculosis Committee (1930), 2-year-old brown trout (Salmo trutta) are the ideal test animal, but frogs may be more conveniently utilized under ordinary laboratory conditions.

#### Technique of Bacteriological Examination

(Furunculosis Committee 1930)

Precautions are taken in obtaining specimens for microscopic examination or artificial cultivation. Fish should be washed with dilute formalin and then with spirit (methyl alcohol) which is allowed to dry before cutting into lesions or viscera. The surface through which an incision is made is also cauterized by a red-hot copper spatula. Cutting instruments, forceps, etc. are sterilized in boiling water or by dry heat. Specimens for film preparations and for inoculating culture media are taken with a wire loop from lesions, heart blood, kidneys, spleen, liver, and intestine. Films are stained by Gram's method or by simple stains, and inoculations are made on plates of nutrient agar. The addition of blood serum to the medium intensifies pigment production by the organism and facilitates its recognition in culture. Plates are

incubated aerobically at 20° C. Colonies of B. salmonicida can be recognized as growth develops by the characters already described, and the formation of a diffusible brown pigment is specially significant. Separate colonies are subcultured onto agar slopes, and their various biological and biochemical characters ascertained.

Pathogenicity can be determined: (1) by inoculating one-fifth of an agar slope culture subcutaneously into a frog; (2) by applying culture to a scarified area of the skin of a brown trout or by injecting it subcutaneously with a syringe. When the animal dies, cultures are made from the heart blood to establish the nature of the infection.

In examining fish which may possibly be carriers of the bacterium, cultures are made from the kidneys, heart blood, and intestine.

In both diseased fish and healthy carriers, the kidney is the organ from which B. salmonicida is most readily isolated (Williamson 1929).

The following diagnostic methods for obtaining specimens from heart blood and kidney have been devised to examine salmon without preliminary dissection or interference with the commercial value of the fish. A 2 cc. syringe is employed, using a needle 3 to 4 inches in length. The syringe and needle are sterilized by boiling for five minutes before taking a sample.

#### Heart blood

The position from which the heart is to be reached is ascertained by the fingers. Actual measurements are useless, owing to variation in size of fish. Pressure is applied on the midventral surface in front of the bases of the pectoral fins, and the finger passed forward until an arch of bone is felt. This is formed by the bones at the base of the gill arches. The wedge-shaped area of soft tissue (isthmus), ending anteriorly in a "V," is formed by the bones and overlies the heart. The area is sterilized superficially by washing with methyl alcohol, and the needle introduced in the midventral line, just in front of the extreme anterior attachment of the pectoral fins. It is easily detected when the wall of the heart is penetrated. The desired amount of blood (one drop is sufficient) is withdrawn by aspiration and placed on nutrient agar medium, on which it is spread with an inoculating needle.

#### Kidney

A point is found on the side of the fish, vertically above the insertion of the pelvic fins, about one-quarter the distance from the lateral line to the midventral surface. This position is chosen as being most suitable, because other viscera are less likely to be injured in

this area. A scale is removed, and the exposed surface is sterilized with methylated spirit. The needle is inserted obliquely and pushed upward and inward, care being taken to avoid the ribs, until it comes in contact with the vertebral column. It is then withdrawn until it is no longer touching the vertebral column, and then the sample is withdrawn. This is mainly blood, but fragments of renal tissue are often also present. The sample is then spread on nutrient agar medium.

The Furunculosis Committee in 1933 supplied additional information for the examination of suspected carriers of B. salmonicida. They emphasized that at autopsy, particular care should be taken to cultivate a sufficiently large amount of kidney tissue. After the body of the fish has been opened and the intestine and air bladder dissected away, a stout inoculating loop is drawn several times through the kidney, breaking up the tissue and mixing it, and then several loopfuls are spread on plates of nutrient agar. When a very large trout or salmon is being examined, a scalpel is used for breaking up the tissue.

The Furunculosis Committee (1933) stated that growth was heavier and more rapid in the presence of tissue fragments or blood. They also stated that the use of fluid media is disadvantageous if the fish has been killed some time before examination, as B. salmonicida grows more slowly than such water organisms that readily invade the blood and kidney shortly after death.

As mentioned in the section on pigment production, lack of pigment production does not necessarily contraindicate B. salmonicida, in view of the factors (pH, temperature, nature of media, and strain of organism) which may determine the time of appearance, and even the presence or absence, of pigment. However, if extremes of pH are avoided, a solid medium selected and the proper incubation temperature (20° C.) used, the danger of missing B. salmonicida on primary isolation is reduced to a minimum.

Two organisms have been reported (Furunculosis Committee 1930) which may be confused with B. salmonicida:

- (1) A short, Gram-negative bacillus which in culture produced a brown pigment like that of B. salmonicida. It differed from B. salmonicida in several respects:
  - (a) motility; (b) abundant growth on potato with a glistening, dirty yellow, and later purplish-brown coloration of the upper part of the medium; (c) fermenting sucrose; and (d) nonpathogenicity to frogs.
- (2) A short, nonmotile, Gram-negative bacillus isolated by Lloyd (1929) from sea water, Firth of Clyde, at 37 fathoms (222 feet). It produced a growth on culture medium which was at first whitish with a bluish or violet tint, but after 15 days yielded a dark brown coloration of the medium. Unlike B. salmonicida, it did not

ferment glucose or mannite and was nonpathogenic to frogs. Though it resembled B. salmonicida in chromogenesis, it was clearly differentiated by its lack of fermentative properties, nonpathogenicity, and by serological tests.

#### COMPARISON WITH ULCER DISEASE

Ulcer disease was the first disease of trout to be studied in the United States. Shortly after its discovery, furunculosis appeared and because of the marked superficial resemblance between the two diseases, much confusion has resulted (Fish 1935).

Ulcer disease was first described by Calkins (1899) who found it present in epidemic form at a hatchery on Long Island, New York. Calkins believed that this disease was caused by a protozoon, but it is now generally recognized that there is little evidence to support this conclusion. Ulcer disease has since been described by many authors (Marsh 1904, Fish 1934, 1935, Wolf 1938, 1940, Davis 1946, Snieszko and Friddle 1949b).

Fish (1934) found that the only organism pathogenic to trout which could be isolated from an outbreak of ulcer disease at a trout hatchery in New York State was Pseudomonas hydrophila. This organism is also the etiological agent in "red-leg" of frogs (Sanarelli 1891, Russel 1898, Emerson and Norris 1905, Kulp and Lackman 1934). Reed and Toner (1941) have also reported it in pike with "red-sore" in eastern Ontario. This organism differs from B. salmonicida in fermentative properties and in the lack of pigment production.

In 1946, Davis stated that a worker in his laboratory isolated a bacterium from fish with ulcer disease, which, on inoculation, produced lesions indistinguishable from typical ulcers of natural infection. This organism had, however, the same cultural characters as Bacterium salmonicida. Brook trout inoculated with this organism by a slight prick with a needle developed a typical epithelial tuft at the site of inoculation (see below). Similar inoculations with standard B. salmonicida cultures failed to produce such a lesion.

On passage through several fish the organism recorded by Davis (1946) increased in virulence and on inoculation into healthy trout produced lesions indistinguishable from those found in typical furunculosis. When first isolated, only superficial lesions were produced by inoculation, and on autopsy, liver, kidney, and spleen appeared normal which is characteristic of ulcer disease (see above). Later inoculations produced deeper lesions more like those of furunculosis and bacteria were found in the kidney, liver, and spleen. There is, therefore, much evidence to support the view that ulcer disease may be caused by a less virulent strain of B. salmonicida.

Snieszko and Friddle (1949b) isolated a new bacterium from an outbreak of ulcer disease among brook trout. This organism was a Gram-negative rod, nonmotile, facultatively anaerobic, but growing better aerobically. It did not liquefy gelatin nor produce changes on litmus milk but fermented some sugars with the production of acid but no gas. It could be propagated only on media containing fresh blood or on sterilized (by filtration) trout tissue extracts.

In several experiments, brook, brown, and rainbow trout fingerlings were inoculated by parenteral injection of pure cultures of this newly isolated bacterium. In all cases, infection occurred with heavy mortality. Since all the brown and brook trout used in the experiments originated from lots having a history of furunculosis, the new bacterium was often accompanied by B. salmonicida. From inoculated rainbow trout, originally free from furunculosis, the new bacterium was regularly reisolated in pure culture.

During an outbreak of ulcer disease among yearling brook trout at the Westfield hatchery (Wisconsin), Flakas (1950a) found predominantly a Gram-positive rod with but few B. salmonicida in lesions. The Gram-positive rod gave the following biochemical reactions: The production of acid in maltose, dextrose, and sucrose, but no change in lactose and mannitol; reduction of nitrate broth; no production indol in peptone broth; no liquefaction in gelatin and the production of a slight amount of acidity in milk. The lesions found on the trout were not like those usually found in furunculosis but like those of ulcer disease, i.e., they possessed a white ulcerous center with a periphery of reddened tissue and were localized in the superficial muscle layers. Those lesions found in experimental trout were also unlike furunculosis.

The exact relationship between these two organisms and ulcer disease is unknown. Flakas (1950a) suggested that ulcer disease may be the result of the action of a bacterium of low virulence, such as the Gram-positive rod isolated, combined with an arrested form of a usually highly virulent organism such as B. salmonicida.

Snieszko and associates (1950a) and Snieszko and Friddle (1950) isolated another organism from brook trout with ulcer disease. This organism fitted best into the genus Hemophilus and they named it H. piscium. In lesions the bacteria occurred as discrete rods with rounded ends and measured 0.5 to 0.7 by 2.0 microns ( $\mu$ ). They stained uniformly or bipolarly with Giemsa stain. In 2-day-old cultures grown on agar slants, cells were 0.8 to 1.0 by 1.0 to 3.0  $\mu$ ; the average dimensions were 0.8 by 2.0  $\mu$ . Cells were arranged singly, in pairs or in irregular groups, and occasionally as filaments up to 12  $\mu$  long. In liquid media cells were of the same size and usually arranged in irregular clusters. They were Gram-negative with Hucker's modification of the Gram method. They did not form endospores and were nonmotile in hanging-drop preparations and in semisolid agar stabs. Capsules were absent with

Tyler's modifications of Anthony's method, but there was evidence of a thin capsule in stained India ink preparations of smooth strains. The organism was found to be a facultative anaerobe, growing best with unrestricted access to air.

The optimum temperature for growth was 20° to 25° C. No growth occurred at 35° C. and was very slight and slow at 7° C. The pH range for growth was from 5.5 to 8.2 with the optimum at about 7.0.

At 20° C., in a liquid medium, the culture remained viable for less than 2 weeks. On agar slants the viability was increased to a period of 5 weeks.

Colonies on agar plates reached a size of about 1 mm. in 2 days and, if well separated, attained a diameter of 2 to 3 mm. within a week. Colonies were circular, smooth, entire, convex, opaque, and cream colored. The colonies of "S" variants grew relatively faster and were butyrous; "R" colonies were compact and tough, and could be removed intact from the medium. Colonies on gelatin plates were of the same appearance. There was no liquefaction.

On agar slant, growth was good within 2 to 3 days. The growth was filiform, slightly glistening, cream colored, butyrous with "S" variants, and brittle with "R" variants. There was no odor and the medium remained unchanged in appearance.

In liquid media, a scant pellicle or ring appeared within a week. Transient clouding occurred within 24 hours and eventually the growth became granular and adhered to the walls, the medium becoming clear. A fine granular sediment was produced. In "S" variants the media remained uniformly clouded for several days.

There was no growth on nutrient agar or in nutrient broth. In gelatin stab cultures, growth was uniform, best near the surface, filiform, and without liquefaction.

Nitrates were not reduced and indole was not formed (Kovac's method). Production of hydrogen sulfide was doubtful, with occasional slightly positive results with lead acetate paper strips. The methyl red test was positive and the Voges-Proskauer test negative after 1 week of growth in Difco V.P. and M.R. medium with fish extract. There was no change in litmus milk and no visible growth on potato, with or without fish extract. There was a slow hydrolysis of starch as indicated with Lugol's solution on starch agar plates. Agar plates with rabbit erythrocytes showed beta-hemolysis.

Two strains of H. piscium were compared for their tolerance to penicillin and streptomycin. H. piscium, strain VII, tolerated 625 units of penicillin but not 1,250, and strain X grew in the presence of 5,000 but not in 10,000 units of penicillin. With streptomycin, the two H. piscium strains gave no growth in the presence of more than 10 units.

The most rapid and intense fermentation took place in a medium containing glucose, fructose, or sucrose. With these sugars the pH fell below 5.0 within 2 to 3 days; no gas was formed. Acid was slowly produced from maltose, trehalose, and starch. Weak and slow production of acid occurred with mannose, galactose, cellobiose, and dextrin. No acid was produced from arabinose, xylose, rhamnose, lactose, melibiose, raffinose, melezitose, inulin, glycerol, adonitol, mannitol, dulcitol, esculin, or inositol.

In media with readily fermentable sugars, growth was rapid and abundant, without the formation of a ring or pellicle. In media with slowly fermented sugars, growth was also abundant, but usually a pellicle or ring appeared within a week. In the absence of fermentation, growth was moderate with slow formation of a ring or pellicle. Without fish extract, there was a very scant growth and acid production in the basic medium with glucose, fructose, mannose, sucrose, trehalose, and cellulose. In all cases the results were uniform, and the smooth variants produced somewhat faster growth and more rapid pH changes in the media.

Under the experimental conditions, the presence of the X and V factors, singly or in combination, was not sufficient for the growth of Hemophilus piscium. It appeared to require some thermolabile substance assumed to be present in potato extract, fish peptone, and rabbit erythrocytes. Rabbit serum would not support growth. Growth was enhanced by the addition of starch to the basal medium containing potato extract or fish peptone.

Repeated bacteriological examinations revealed that in the infected trout, H. piscium seems to be confined to the ulcer; it can be found in the internal organs, as the blood and kidney, only in the advanced stages of the disease. E. salmonicida, on the contrary, is present in the kidney and blood from the earliest stage of the infection. Additional pathogenicity tests were conducted on tadpoles and bluegill sunfish. All inoculated tadpoles and sunfish survived, indicating that the H. piscium was pathogenic to trout only.

Since several different organisms have been reported from fish with ulcer disease, the evidence therefore suggests that this disease can be caused by a number of different bacteria which behave in fish in a similar manner. The predominating organism of ulcer disease may vary with the individual hatchery or even with broad geographical regions. Snieszko and associates (1951) for example, have shown that H. piscium is the causative agent in the eastern United States. The

suggestion is therefore put forth that ulcer disease may be regarded as a clinical entity caused by any one or more of a number of different organisms, rather than a disease consistently produced by a specific organism. Furunculosis, on the other hand, is brought about by a specific organism, B. salmonicida.

The symptoms and pathology of ulcer disease also differ in several respects from those of furunculosis (see below). However, if B. salmonicida is found on occasion to produce lesions suggesting ulcer disease, as has been reported (Davis 1946), it would perhaps be better to refer to the disease as furunculosis. When determining whether or not ulcer disease is present, the symptoms and pathology would seem to be the important aspect of the disease, the organism being of secondary importance except when B. salmonicida is present. In the case of furunculosis, B. salmonicida must be present to produce this disease, the symptoms and pathology receiving somewhat less emphasis accordingly. In differentiating between ulcer disease and furunculosis, these distinctions would seem logical in the light of our knowledge at the present time.

#### Pathology

The first recognizable symptoms of ulcer disease are raised, somewhat tufted, white spots consisting of fine shreds of superficial skin layers which Fish (1935) named "epithelial tufts". These tufts may appear on any part of the body, even on fins, but in most cases the first ones are found on the body proper. They are first noticed when about 1 mm. in diameter and may be as much as 3 or 4 mm. across before the corium is penetrated and a recognizable ulcer is formed. A casual inspection might lead to the conclusion that these tufts are small patches of fungus (Wolf 1938).

In 1934 Fish called attention to the fact that the pathology of ulcer disease differs from furunculosis in several important points. Borderline cases may be found, however, particularly in fingerling trout which are exceedingly hard to diagnose accurately without resorting to bacteriological examination.

In the first place, with ulcer disease the lesions are clear-cut, the whole picture being typical of a sloughing necrosis progressing from the external surface inward (Fish 1934). According to Wolf (1938), exposed muscle tissue often appears firm and healthy, but he states that this is by no means constant, as inflammation may extend for some distance around the ulcer.

Secondly, the contents of the lesions of ulcer disease are usually light in color and easily washed away, leaving the grayish-white muscular tissue (of trout) fully exposed (Davis 1946). Furunculosis is an internal infection which moves towards the surface and produces

subcutaneous lesions which eventually perforate the epithelium. The ulcers so formed have ragged and irregular margins and are deep red in color, owing to the large accumulation of blood and liquefied tissue (Fish 1935).

A characteristic of ulcer disease is that the edges of the jaws and the roof of the mouth are very often attacked (Wolf 1938, Davis 1946). All the soft tissue over a large area of the roof of the mouth may be eaten away and ulcers at the edges of the jaws often progress very rapidly, especially on the lower jaw where the bones may be eaten through (Wolf 1938).

Small ulcers frequently develop on the fins in ulcer disease and these attack the fin rays as well as the soft tissue. Often a group of rays is punctured midway, or even closer to the base of the fin (Wolf 1938). Fish (1934) was unable to culture any bacteria from the blood or internal organs of fish with ulcer disease, which is not the case with furunculosis, as is well-known.

The occurrence of fungus on lesions of ulcer disease is rarer than with furunculosis. Fish (1934) stated that the lesions of ulcer disease are seldom, if ever, attacked by fungus during the life of the host, whereas those of furunculosis usually support a luxuriant growth of Saprolegnia. Wolf (1938), however, did obtain several fish at various times from hatcheries which had considerable fungus.

Fish (1934) believes that the etiological agent is capable of producing a toxin which may be absorbed from lesions and may in part account for the heavy mortality accompanying the disease. This tentative conclusion is based upon the large amount of microscopical necrosis surrounding a relatively small accumulation of bacteria. Microscopic areas of necrosis have even been found in the liver and kidney of heavily infected hosts sacrificed at the point of death. These areas showed no evidence of bacterial invasion or cellular infiltration. As already pointed out there has been no suggestion of a toxin with furunculosis (Furunculosis Committee 1935).

It seems quite probable that the primary site of infection in ulcer disease is at a point of injury to the normal epithelium (Fish 1934). Whether or not the causative agent is capable of independent penetration of the epithelium is not known. The first protective response to the infection is a marked thickening of the epithelium, which is responsible for the appearance of the epithelial tufts and the white line of fin lesions. At this time, the bacteria may be found in a localized area between the base of the epithelium and the subepithelial connective tissue. A slight infiltration of polymorphonuclear leucocytes is noticeable at this stage. A progressive necrosis extends in all directions from this picture which results in complete disintegration and sloughing of the scales and subepithelial connective tissue. A

marked infiltration of leucocytes is then noticeable in the tissues surrounding lesions. This leucocytic response definitely differentiates ulcer disease from furunculosis in which there is no such outstanding infiltration.

The heavy subcutaneous tissue band forming the corium of the skin apparently offers very little resistance to the bacteria and sooner or later is penetrated exposing the skeletal musculature which becomes markedly hyalinized. Bacteria continue to grow in the accumulation of necrotic debris resulting from the death and subsequent destruction of muscle bundles (Fish 1934). Davis (1946) contradicts Fish (1934) on this point, saying that there is little evidence of undermining of deeper muscles so characteristic of furunculosis. A possible truth of the matter may be that the underlying musculature may or may not be destroyed according to the virulence of the organism and susceptibility of the host.

Recently, Snieszko and associates (1951) have reported that terramycin is effective in the treatment of ulcer disease in brook trout. They found that the proportion of surviving trout depended on the rate of mortality at the onset of treatment; if this was not too high, results were good. Aureomycin had no therapeutic value. Sulfonamides have previously been shown to be unsatisfactory (Tunison and McCay 1937, Wolf 1939 and others).

#### CONTROL

Within recent years, with the increase in production of legal-sized trout at a comparatively high cost, the necessity of preventing and controlling fish diseases has assumed major importance. Our knowledge of the methods of prevention and elimination of fish diseases has increased considerably but there is still much to be learned. Some diseases are controlled quite easily, while others, such as furunculosis, are more difficult. The only known method of absolute control of B. salmonicida infection involves complete elimination of all fish from a hatchery, thorough disinfection of the hatchery, the rebuilding of a new stock of disease-free fish, and the maintenance of disease-free conditions throughout all future operations. Unfortunately, this method can only be used at those hatcheries having a controlled water supply, that is, originating in wells or springs that can be kept free from fish (O'Donnell 1944).

The measures for the control of furunculosis fall into two groups: (1) the prevention of epidemics, and (2) handling an epidemic which has already started.

## Prevention

### In hatcheries

#### Care of Ponds and Utensils

In the care of ponds, troughs and raceways arrangements should be made, wherever possible, to disinfect them thoroughly at least once a year. This procedure is a "must" after a severe epidemic and disinfection should begin at the upper waters first. Chlorine, because of its efficiency in killing pathogenic organisms and of its low cost has been found to be the most desirable chemical disinfectant for hatchery use (Davis 1938, Fish 1939, Connell 1939, Hagen 1940, Wolf 1940, O'Donnell 1944). Before chlorination is begun all fish should be removed. Chlorine is available in powder or fluid form which liberates various amounts of "available" chlorine.

One of the best papers on the disinfection and maintenance of hatcheries is that written by O'Donnell (1944) and for more complete treatment of this subject the reader should consult that paper as only general procedures will be given here. The concentration of chlorine found by O'Donnell (1944) to be most effective in all hatchery work is 200 p.p.m. When clean surfaces are kept moist with such a concentration, O'Donnell (1944) found that disease producing organisms were killed in less than 30 minutes. Although it is desirable to maintain the solution at full strength, occasionally this is impossible, such as in the treatment of raceways, owing to dilution, but under no conditions should the concentration fall below 100 p.p.m. according to O'Donnell (1944). At 100 p.p.m. disease producing organisms are killed in one hour or less. O'Donnell (1944) says that mud, muck and other organic material dissipates chlorine at a rapid rate and as much of such material as possible should be removed. Before starting the actual chemical disinfection of the hatchery, a number of preliminary operations are necessary. All ponds, raceways, and troughs should be measured for capacity and calculations made of floor surfaces in the buildings, allowing for 3 inches of solution on all floors. After determining the type of chlorine solution or powder to be used, calculations are made of the quantity needed to produce a concentration of 200 p.p.m. In the event that the chlorine solution runs into fish-bearing waters after leaving the hatchery, neutralization will be necessary to render the chlorine inactive and harmless. Commercial sodium thiosulphate ("hypo") is used as a neutralizer, 5.6 grams being required to neutralize each gallon of 200 p.p.m. chlorine solution.

Before adding chlorine, all ponds, raceways, and troughs should be drained. Davis (1938) suggested allowing ponds to dry in the sun for several days. Additional splash boards are to be provided in certain sections to allow raising of the water to the top of each section. After draining, the splash boards are installed and water is allowed to rise until the particular section is about half filled. Then one-half of the calculated amount of concentrated chlorine is emptied into the raceways,

and stirred thoroughly by dragging a loose coil of chicken-wire back and forth along the bottom. When the water has risen within 2 inches of the top of the splash boards, the remaining chlorine concentrate is emptied into the section. The solution is again stirred with chicken-wire. The same general procedure is continued until the entire raceway system, including all springs, is completely filled with water containing chlorine. The operation of a small outboard motor in a raceway is helpful in circulating the chlorine solution.

O'Donnell (1944) also gave detailed instructions for treating the inside of hatchery buildings, and the reader should consult his paper for these instructions. All loose equipment should be brought out of storage and scrubbed thoroughly with warm water and soap, if possible, and left near a raceway for chemical disinfection later. Such equipment includes buckets, pans, small troughs, tubs, end screens, seines, extra splashboards, and many others. During this operation much worn-out equipment may be found and destroyed. Hatching and rearing troughs should be scrubbed clean. The side walls of all raceways should be scrubbed and the bottoms raked. Particular attention should be given to any left-over fish food, pond scum, or any other substance of similar nature.

It is also stressed by this author that after the hatchery is completely disinfected and disease-free, the prevention of recontamination is of prime importance. The movement of any live fish into the hatchery should be absolutely forbidden and production should be started with disinfected eggs. All eggs which are received should be disinfected immediately (see below). The hatchery must be protected from the water and packing cases of shipped eggs, the hands of handlers of such eggs, and from the shipping truck. Workers' hands should be cleaned with soap at intervals. All trucks and equipment coming into the hatchery should be disinfected on the road before entering the hatchery. Drivers should not be allowed to assist in loading or even to visit any part of the hatchery.

#### Handling of Eggs

Since the Furunculosis Committee (1930) isolated Bacterium salmonicida from the ovaries of infected trout, it has been obvious that eggs may serve as carriers of this organism from the infected brood stock to freshly hatched fry. Fortunately, B. salmonicida has been found only on the surface of healthy eggs (Smith 1939), while the contents of normal trout eggs were found to be free from infection (Williamson 1929, Furunculosis Committee 1930). Ova may be contaminated before extrusion, if the female parent is infected, or afterwards by contact with contaminated implements, water or hands of workers who have been dealing with infected fish (Blake 1930).

The surface disinfection of fish eggs is only practicable with a disinfectant which is non-toxic to the eggs in a concentration permitting a fairly rapid destruction of B. salmonicida or other pathogenic bacteria

that might be carried over the surface (Snieszko and Friddle 1948). On the other hand, the action of the disinfectant should not be affected by the presence of proteins (Snieszko and Friddle 1948).

Many chemicals have been tried as external disinfectants of trout eggs and Gee and Sarles (1942) listed a number of them in the order in which they proved useful. The solution percentages given are for use in 10-minute treatments of eggs.

<u>Disinfectant</u>	<u>Percent solution</u>
Recommended:	
1. Sulfo-merthiolate (Lilly).....	0.015
2. Acriflavine .....	0.185
Safe:	
3. Mercuric chloride .....	0.0025
4. Mercurochrome .....	0.68
5. Chlorozene (Abbott) .....	0.009
6. Mercarbolide (Upjohn) .....	0.0052
7. Calcium hypochlorite (B-K powder) .....	0.026
Usable but dangerous:	
8. Iodine .....	0.0056
9. Azochloramid (Wallace & Tiernan) .....	0.001
10. Gentian Violet .....	0.15
11. Sodium hypochlorite (Klenzade) .....	0.062
Extremely dangerous:	
12. Formaldehyde .....	0.53
13. Metaphen .....	0.0099
Not usable:	
14. Glymocol	
15. Phenol	
16. Hexylresorcinol	
17. Malachite green	
18. Brilliant green	
19. Potassium permanganate	

The list shown above (Gee and Sarles 1942) indicates clearly that the best trout egg disinfectants are sulfo-merthiolate and acriflavine. Therefore, some space will be devoted to their general application, but the author does not feel it necessary to deal in detail with the techniques

associated with the disinfection of trout eggs as these are readily accessible (see particularly The Progressive Fish-Culturist) and are being revised from time to time.

Acriflavine has been suggested by several authors as a suitable disinfectant of trout eggs (Blake 1930, Atkinson 1932, Furunculosis Committee 1933, Smith 1939, 1940, 1942, 1944, O'Donnell 1944). Eggerth (1926) showed that the bactericidal action of acriflavine was greatly reduced in neutral and acid solution. Smith (1942) found that only those solutions (500 p.p.m.) at pH 7.0 and above killed B. salmonicida in 20 minutes and at pH 5.5 the bacteria survived for over 50 minutes. By using acriflavine (500 p.p.m.) at pH 7.7, Smith (1942) found the organism was killed in less than 20 minutes. No abnormalities in fish resulted from this treatment (Smith 1942). Smith (1944) gathered data which indicated that for B. salmonicida organisms in the lag phase and logarithmic death phase are more resistant to the action of 500 p.p.m. alkaline acriflavine solution at 1° C. than are bacteria in the phase of logarithmic growth.

The recommended dilution of acriflavine is 1:2000 (approximately 500 p.p.m.) (Blake 1930, Atkinson 1932, Furunculosis Committee 1933, Smith 1940, O'Donnell 1944). Either eyed or "green" ova may be disinfected with safety, but with "green" ova treatment should be carried out on the day of fertilization, or a few hours after that process is completed (Blake 1930).

Ova to be treated should first be washed with clean water, then drained and placed in suitable vessels such as enamelled basins (Blake 1930). A solution of acriflavine (1:2000) in well-aerated water should be poured over them in the proportion of at least three and a half fluid ounces to each 1000 ova. The quantity of germicide does not increase the mortality among ova and the volume mentioned may be increased without danger, but it should not be diminished, or there will not be sufficient to ensure disinfection. The disinfectant should be left in contact with the ova for 20 minutes as a minimum and treatment may be prolonged to 30 minutes (Blake 1930, O'Donnell 1944). After treatment, ova should be washed in clean water.

Information regarding precautions to be observed while disinfecting trout eggs and regarding the necessary details for the disinfection of eggs with acriflavine, is given in a recent article by O'Donnell (1944). Snieszko and Friddle (1948) also give instructions on cleanliness while disinfecting trout eggs.

Turning to sulfo-merthiolate Gee and Sarles (1942) found that better results were obtained with this germicide than with acriflavine. Snieszko and Friddle (1948) showed that trout eggs in all stages of development could be disinfected safely with sulfo-merthiolate.

Because eggs are tender during the early stages of development and require extremely careful manipulation, it is highly desirable that disinfection with sulfo-merthiolate be effected either very soon after the eggs are water-hardened or after they are eyed. Eggs not in the tender stage can be disinfected in several ways: (1) they can be poured into a pan or tub with a solution of sulfo-merthiolate and stirred with a feather or by hand during disinfection. After disinfection, they should be drained and placed in fresh water. (2) Eggs can be distributed in hatching trays and then immersed with the trays in the disinfectant. After disinfection, they can be placed in the hatching troughs without rinsing (Snieszko and Friddle 1948).

According to Gee and Sarles (1942) sulfo-merthiolate in dilution of 1:7500 kills E. salmonicida in the presence of trout eggs within 10 minutes. As this drug is not toxic to trout eggs even in much lower dilutions, 1:5000 dilution gives better assurance of thorough disinfection. In whichever manner eggs are disinfected, they should be exposed to the action of the disinfectant for no less than 10 minutes. During that time eggs, except in the sensitive stage, should be moved at least once every 2 minutes to expose all surfaces to the disinfectant (Snieszko and Friddle 1948).

As many as 50,000 eggs can be disinfected in 10 liters of disinfectant solution. Several small batches of eggs can be treated in the same batch of sulfo-merthiolate, providing that the solution is well aerated and not more than a few days old. Sulfo-merthiolate does not attack metal, but because even traces of zinc are toxic to fish, it is safer to dip eggs in a painted enamelled or glass vessel. This drug should never be exposed to direct sunlight (Snieszko and Friddle 1948).

Regardless of the disinfectant used, all damaged, clouded, and spotted eggs should be discarded and destroyed as E. salmonicida or other pathogenic bacteria may be within the eggs, and these organisms might not be reached and destroyed (Snieszko and Friddle 1948).

#### Care of Fish-Immunization and Selective Breeders

Besides the construction of hatcheries designed to reduce the possibility of disease transmission, disease prevention requires the establishment of conditions which will maintain the vitality of fish (Belding 1927).

In order to maintain a resistant stock of trout Belding (1927) suggested the following rules:

- (1) Do not crowd fish. Keep the stock below the maximum number which may be accommodated.
- (2) Do not overfeed. Use no raw food.

(3) Maintain a moderately low temperature.

(4) The water supply should permit the mixing of warm and cold water so that any desired temperature can be maintained.

(5) Pools should be constructed with emergency outlets and inlets so that each pool can function as an independent unit.

(6) Pool construction should permit easy cleansing and sterilizing.

In general immunization of trout against furunculosis is neither particularly successful (Snieszko and Friddle 1949a) nor simple to carry out (Duff 1942). Duff (1942) did obtain some fairly good results but the work of Snieszko and Friddle (1949) would indicate that consistency can not be expected from this procedure.

The results of Duff (1942) showed that, in the case of the addition of live virulent culture to water at approximately 19° C., prolonged prior oral vaccination decreased the mortality owing to an initial infection from 75 percent to 24 percent in yearling cutthroat (Salmo clarkii) and 71 percent to 26 percent in tests in which yearling and 2-year-old fish were included. It is therefore only possible to conclude, on the basis of a total of 267 trout involved that only one heavy infection produces a mortality of roughly 75 percent in normal fish, compared with roughly 25 percent in vaccinated fish.

When vaccinated fish were infected by intramuscular inoculation, mortalities were higher than by the method of adding virulent culture or infected fish to water (Duff 1942). The results of Snieszko and Friddle (1949a) also indicated that oral immunization did not increase immunity sufficiently to protect brook trout (Salvelinus fontinalis) from bacteria introduced parenterally in quantities capable of producing an acute infection.

Duff (1942) found a marked increase, in the vaccinated groups, in the number of fish showing specific agglutinins and a tendency in this group toward higher agglutinin-titres.

Embody and Hayford (1925) and the Furunculosis Committee (1930) suggested the selective breeding of a resistant race to furunculosis. Embody and Hayford (1925) found that in the course of three generations of selective breeding there was an increase in the average resistance of trout to bacterial diseases. This was indicated in the decrease from 98 percent mortality, in the eyed stage to fingerlings, to 30.8 percent in the same age group three generations later. Selection was made from survivors of various diseases after the diseases had run their courses. Although comparatively little work has been done on this aspect of control of furunculosis, the author feels that there is much to be said for it, in the light of the work of Embody and Hayford (1925).

## In nature

The prevention of furunculosis in nature can be summed up in four suggestions:

- (1) Avoid stocking rivers and streams with fish known to be infected.
- (2) As far as possible, keep water levels of rivers up.
- (3) Avoid pollution of rivers and streams, particularly organic pollution.
- (4) If an epizootic is in progress, to help prevent further spread of infection remove dead and diseased fish. Bury all dead fish.

## Treatment

In the past there has been no curative treatment or means of preventing the spread of furunculosis through any pond or trough among which it has appeared. Where it has spread through a hatchery, the only sure way to get rid of furunculosis has been to remove all fish, disinfect all ponds and troughs, and restock with healthy fish (Gutsell 1947).

With the advent of sulfonamides there has been a marked change in this picture and a number of workers have experimented with this group of drugs in the hope of effecting a cure (Gutsell 1946, 1946a, 1948, Wolf 1947, Gutsell and Snieszko 1948a, 1948b, Snieszko and Friddle 1949b, and others).

## The best drug

To date, sulfamerazine has been found to be the best drug in the treatment of furunculosis (Gutsell 1945, Snieszko and associates 1950b). In 1945 Gutsell experimented with five drugs on fingerling brook trout (8 months) with furunculosis. These five were: (1) sulfamerazine, (2) sulfathiazole, (3) sulfanilamide, (4) sulfadiazine, and (5) furacin, each administered by being mixed with food. In another treatment furacin was added to the water of troughs. Furacin in the water was not beneficial but furacin in food had some helpful effect. Results with sulfanilamide and sulfadiazine were not encouraging. Sulfathiazole was more beneficial than furacin, but decidedly less effective than sulfamerazine.

The improvement with sulfamerazine was impressive. Mortality dropped readily within a few days, generally was light after a week, and within 2 weeks almost completely stopped. Through 25 days the loss was 17 percent as compared with 50 percent among infected lots not receiving medication.

Snieszko and associates (1950b) tried another sulfonamide, sulfamethazine, which showed considerable promise. Although definitely not more effective than sulfamerazine in yearling brook trout with furunculosis, it was slightly so with fingerlings. In spite of these results authors still consider sulfamerazine to be the best drug in an acute epizootic.

Sulfonamide therapy has been found to be very much more effective in the treatment of fingerling brook trout with furunculosis than with yearling brook trout (Snieszko and associates 1950b). Wolf (1947) found that sulfamerazine was decidedly beneficial in the treatment of furunculosis in lake trout and brown trout.

#### Toxicity and tissue levels of sulfonamides in fish

Pharmacological investigations of sulfonamides in fish were first carried out by Litchfield in 1939. Of a number of sulfonamides sulfathiazole has been shown to be the least toxic, although unfortunately, as already indicated, one of the least beneficial in the treatment of furunculosis. Smith and Nigrelli (1947) carried out toxicity tests with sulfathiazole, sulfadiazine, sulfamerazine, and sulfamethazine. They found in one experiment using Tilapia macrocephala (bleeker) as a test fish, that the non-toxic concentration for sulfathiazole was 0.1 percent, for sulfadiazine and sulfamerazine 0.5 percent and for sulfamethazine, it was 0.14 percent. In a second experiment, five fish were placed in 20-gallon tanks containing 62 liters of water. The drug concentration was 0.8 mg. per cc. This concentration remained more or less constant throughout the experiment. For sulfamethazine a toxic reaction was obtained in 16 days; for sulfamerazine in 11 days; for sulfadiazine in 20 days; and no toxic effects were obtained with sulfathiazole. Except for sulfathiazole, therefore, all other sulfonamides used were toxic at this concentration. Changes in pH were not found to be a factor in these toxicity tests as there were no deaths at the non-toxic levels even though there was a change in pH.

It has been shown (Gutsell and Snieszko 1949b) that sulfamerazine is not lethal to trout. Given sulfamerazine at daily dosage rates of 5, 10, and 15 gm. per 100 pounds of fish per day, these authors found that in the case of brook trout and rainbow trout, mortalities were as low in groups treated at the highest rate as in any lot. Moreover, mortalities did not increase towards the end of the treatments. Similarly, with brown trout deaths could not be attributed to sulfamerazine poisoning when treated at these rates.

Where there was evidence of growth retardation, consumption of sulfamerazine-treated food was poor. When consumption of treated food was good, growth was equal or nearly equal to that in controls. The apparent explanation of reduced food consumption when sulfamerazine was mixed with food was that certain species of trout (especially brown

trout) dislike the taste of the drug. Growth of rainbow trout did not appear to be affected and brook trout showed marked retardation only at the 10- and 15-gm. dosage rates. On the other hand, food consumption was very poor and growth stopped in brown trout treated with sulfamerazine at any rate. In 1947, Gutsell did find that a 10 gm. rate or more slowed growth in fingerling brook trout but that little effect was observed in rainbow trout even at the 15-gm. rate.

Hemoglobin content and erythrocyte counts have been found to increase in brown trout treated with sulfamerazine (Gutsell and Snieszko 1949b). For fish treated at 5-, 10-, and 15-gm. rates, the hemoglobin content was 9.7, 9.4, and 8.9 gm. respectively. For control fish the content for 100 cc. of blood was 7.0 gm. The level of hemoglobin for fish treated at the 5-gm. rate was significantly higher than that for the 15-gm. rate but not so for 10-gm. rate. Erythrocyte counts made after 42 or more days on brown trout showed mean counts per cubic mm. of 1,009,500 in the controls, 1,441,000 for the 5-gm. rate, 1,265,250 for the 10-gm. rate, and 1,300,000 for the 15-gm. rate. Significance was shown only between the 5- and 10-gm. rate.

Gutsell and Snieszko (1949b) pointed out that the increase of hemoglobin and of erythrocytes in fish treated with sulfamerazine may have several explanations. It is possible that some infection causing a moderate anemia was cured or greatly reduced by sulfamerazine therapy. Another hypothesis is based on the finding that sulfonamides inhibit respiratory enzymes (Altman 1946). It is possible that this action has the same effect as a reduction in the supply of oxygen and like it results in higher erythrocyte and hemoglobin levels (Phillips et al 1947). A final possibility is that sulfamerazine actually stimulates the production of hemoglobin and erythrocytes in trout.

Snieszko and Friddle (1951) have shown that increase in dosage of sulfamerazine results in a proportional increase of tissue concentration of this drug. Using brook trout (Salvelinus fontinalis) weighing about 23 gm. each, it was found that maximum tissue concentrations were attained on about the sixth day. These were approximately 3.7 mg. percent for a 10 gm. dosage per 100 gm. of trout per day, 6.9 mg. percent for a 20 gm. rate, and 8.9 mg. percent for a 30 mg. rate. The tissue concentration of sulfamerazine was also found to increase during the first 4 days. It was then relatively uniform during the next 6 days but dropped rapidly when the drug was eliminated from food on the 10th day (to less than 0.25 mg. percent by the fourteenth day).

Of eight sulfonamides administered to brook trout (S. fontinalis), Snieszko and Friddle (1951) found that sulfanilamide reached the highest tissue concentration (8 to 9 mg. percent). The concentration began to drop after the ninth day regardless of continued treatment. The reason for this is unknown. The tissue concentration of sulfamerazine reached a somewhat lower level than that of sulfamethazine but the

absorption of the former was faster. Sulfaguanidine was absorbed more slowly and reached a lower concentration than sulfadiazine and both attained lower levels than either sulfamerazine or sulfamethazine. Sulfathiazole was only slightly absorbed. Sulfathalidine and sulfaquinoxaline were not taken up from the intestinal tract. All sulfonamides were given to fingerlings weighing 2.5 to 4.0 gm. at a dose rate of 20 mg. per 100 gm. of fish per day.

The concentration of sulfamerazine in yearling brook trout (S. fontinalis) increases most rapidly and reaches the highest levels in blood and liver (Snieszko and Friddle 1951). These were found to be slightly over 25 mg. percent by the ninth day for blood and about 22 mg. percent by the same day in the case of liver. For these experiments sulfamerazine was given at the rate of 20 mg. per 100 gm. of fish per day. Results for kidney and muscle were approximately 12.5 and 9 mg. percent, respectively. The concentration of the drug increased rapidly up to the ninth day but then dropped sharply even though the dosage was continued at the same rate. In the gut, the drug concentration remained well over 100 mg. percent during treatment. This indicates that either sulfamerazine is accumulated in the alimentary tract or is excreted until some balance is achieved between the concentration in blood and the intestine. When the diet containing sulfamerazine was discontinued (after the thirteenth day), the concentration of the drug decreased uniformly in blood and other tissues.

The tissue concentrations of sulfamerazine given at constant rates have been found to be higher in older trout than in younger trout, even though the former were fed at lower rates. Whether this is related to age or not, can only be determined by further experiments (Snieszko and Friddle 1951).

To test the effect of high initial doses Snieszko and Friddle (1951) treated brook trout fingerlings (S. fontinalis) with sulfamerazine at first at the relatively high rate of 30 mg. per 100 gm. of fish per day. The tissue concentration slowly rose until it began to level off on the 4th day at 9 mg. percent. From the seventh day, fish in each of three groups received sulfamerazine at different rates of 30, 20, and 10 mg. percent. In those receiving 10 and 20 mg. daily doses, the tissue level dropped considerably within several days. However, it was only slightly higher than in trout used in other experiments in which fishes were treated with identical dosages but without the benefit of the high initial doses. The only advantage of high initial dosage in trout was the somewhat shortened time with which the higher tissue level was reached. The subsequent lower doses did not permit the maintenance of the high tissue concentration obtained by the high initial dosage.

#### Dosage, duration of treatment and final recommendations

In the treatment of trout for furunculosis, it is essential that a relatively high initial dose of sulfonamide be given. Evidence from recent work (Flakas 1950b) would indicate that when B. salmonicida enters

the tissues of a brown trout possessing a blood-sulfonamide level which is nonprotective, this pathogen can remain in tissues in a state of temporary arrested virulence. The state of arrested virulence can continue to exist despite the subsequent establishment of a "protective" blood-sulfonamide level. Consequently, an active infection could develop when the blood-sulfonamide level drops to a nonprotective value. On the other hand, when B. salmonicida enters the tissues of a brown trout possessing a protective blood-sulfonamide level, the organism can be retained in the host without producing an active infection. It was observed that active infection did not develop even after the blood level had dropped below the protective range (found to be below 7 mg. per 100 ml. of blood).

Satisfactory therapeutic results were obtained by Gutsell and Snieszko (1949a) with dosages of 8.8 and 13.4 mg. per 100 gm. of trout per day (4 and 6 gm., respectively, of sulfamerazine per 100 pounds of fish per day). Flakas (1950b) found that the therapeutically effective dosage of sulfamerazine in the treatment of furunculosis in adult brown trout was 17.6 mg. per 100 gm. of fish per day (8 gm. of drug per 100 pounds of fish per day). After 4 days of such treatment, the blood level rose to 7 mg. percent.

Results of Snieszko and Friddle (1951) indicate that absorption of those sulfonamides which are most effective in the treatment of furunculosis is somewhat slower in fishes than in warm-blooded animals (Litchfield 1939). In order to bring about a fast, therapeutically effective blood concentration of sulfamerazine, trout should receive during the first 3 to 4 days of treatment 20 to 30 gm. of the drug per 100 kilograms of fish per day (10 to 15 gm. per 100 pounds). After this initial period, dosage can be reduced by 25 to 50 percent without lowering the blood concentration of sulfonamides below the minimum effective level.

According to Flakas (1950b), about one-third of sulfamerazine should be replaced by sulfaguanidine. Sulfaguanidine exerts its effect, for the most part, in the intestinal tract and since the intestinal tract of trout carries B. salmonicida during active infection, this affords a good source of infection by which the disease may be spread. Flakas (1950b) felt that sulfamerazine alone was not totally effective as a means of ridding a trout hatchery of B. salmonicida.

Gutsell and Snieszko (1949a) and Snieszko and Friddle (1951) outlined the following general recommendations for sulfonamide treatment of furunculosis, and possibly other infectious diseases of fishes:

1. Treatment should be started as early as possible, because fish in the advanced stage of the disease cannot be saved.
2. Bacteriological examination of diseased fish should be carried out as soon as possible. Other diseases, which in their

symptoms may be similar to furunculosis, may be refractory to sulfonamide treatment. In case of mixed infections in which B. salmonicida is also present, treatment with sulfonamides should be given.

3. Treatment should be started before the results of bacteriological examination are known. If bacteriological examination reveals infection with pathogens which are known to be refractory to sulfonamides, treatment should be discontinued and other measures tried.

4. Mix sulfonamides very thoroughly with food of good cohesion. Good practice is to mix sulfamerazine with dry feed (containing 4 percent salt) before it is mixed with spleen and water. A satisfactory ratio of meat, dry feed, and water is 2:2:1 by weight.

5. The effectiveness of treatment can be increased by removal as often as practicable of all dead, sick, and runt fish which are less likely to take adequate quantities of medicated food.

6. Treatment should be repeated if the disease reappears. Very prolonged treatment, or treatment with inadequate dosage of drug may result in the appearance of drug-fast strains of micro-organisms.

7. Give fish plenty of food--about all they will take, but no more.

8. Keep pools and troughs clean and observe strictly all sanitary measures practiced in the control of infectious diseases.

#### SUMMARY

1. Furunculosis was first described by Emmerich and Weibel (1894), who found the causative agent to be Bacterium salmonicida. Since then, the disease has been found widespread throughout Europe and the United States.

2. Numerous bacterial diseases of fish were described before 1894 but from the literature only can the general nature of these be recognized.

3. B. salmonicida is a short cylindrical organism, Gram-negative, nonmotile, non-spore-forming with a tendency towards pleomorphism.

4. The temperature range for growth of B. salmonicida is between 5° and 30° or 32° C. The optimum growth temperature is stated to be from 10° to 22° C. The organism has been recovered from fresh water for up to 2 weeks. It is short-lived in salt water. The organism survives longer in sewage material free from trade wastes (up to 67 days). The organism is an obligate parasite.

5. B. salmonicida is aerobic and facultatively anaerobic, grows well on agar and in broth, but poorly on potato. It produces a brown pigment on culture media only under aerobic conditions.

6. The organism liquefies gelatin and serum media. It does not produce indole but ferments glucose, mannite, galactose, levulose, maltose, and salicin. Sucrose, lactose, dulcitol, and raffinose are not fermented.

7. The organism gives rise to complement fixation and agglutination with specific antisera.

8. Smooth (S), rough (R), and G-type colonies of B. salmonicida have been demonstrated. The smooth type organism is pathogenic and rough nonpathogenic. The organism falls into two groups with respect to R and S phases: Group I,  $R = S$  plus  $n$ ; Group II,  $S_1 = R_1$  plus  $n_1$ .

9. A bacteriophage has been demonstrated, but it is difficult to correlate it with the distribution of the disease. It has been associated with infected organs of fish and is quite specific. Its origin is obscure.

10. The disease is a general infection, affecting the kidney, spleen, liver, and muscular tissues. There may be congestion of blood vessels in the abdominal cavity and a discharge of blood and mucus from the vent. Typical lesions are areas of necrosis. No active toxin has been demonstrated, but the organism is proteolytic. Leucocytic infiltration is not outstanding in furunculosis.

11. In the Salmonidae the order of susceptibility is brown trout, brook trout, and rainbow trout. Many other fresh water fish are also susceptible.

12. The disease is spread by water and food. Carriers of the disease are an important reservoir. Migratory Salmonidae, however, are not thought to be important in the spread of the disease.

13. High temperature and low water levels favor the prevalence of the disease. The temperature must be within  $55^{\circ}$  to  $66^{\circ}$  F. before an important epizootic will take place in nature. The role of water pollution in the spread of the disease is undecided.

14. Diagnosis depends on culturing the causative organism. Confirmation may be obtained by injecting the organism into a fish or frog.

15. Furunculosis differs from ulcer disease in several respects. The lesions of ulcer disease are clear-cut, and their contents easily washed away. Ulcers of furunculosis are ragged and deep red in color.

In ulcer disease the jaws and roof of the mouth are frequently attacked. Fish (1934) was unable to demonstrate any bacteria in blood and internal organs of fish with ulcer disease. He believes the causative agent produces a toxin. Leucocytic infiltration is outstanding in ulcer disease. Fungus on lesions of ulcer disease is rarer than in furunculosis.

16. Control of furunculosis falls under two categories, prevention and treatment. Hatcheries should be disinfected at least twice a year, using a concentration of chlorine of 200 p.p.m. Utensils and workers' hands should be cleaned regularly. All eggs should be disinfected with either acriflavine (1:2000 for 20 minutes) or sulfo-merthiolate (1:7500 for at least 10 minutes). Immunization of trout is not particularly successful, but the results of selective breeders are encouraging. Prevention in nature amounts to stocking with healthy fish, keeping water levels up, avoidance of pollution, and burying all dead fish during an epizootic.

17. The best drug in the treatment of furunculosis is sulfamerazine. The order of toxicity of various sulfonamides is: sulfamerazine, sulfamethazine, sulfadiazine, and sulfathiazole, which is least toxic and least satisfactory for treatment. Dosages and duration of treatment and final recommendations are discussed.

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It is hoped that this little monograph, which is based entirely on information gathered from the literature, as well as the list of 189 references, will prove useful to those studying Furunculosis of Fish.

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