accepted for Dept. of Bacteriology

Prof & Head of Dept of Bact. Apr 26 1932

STUDIES ON B. SALMONICIDA

By

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A Thesis submitted for the Degree of
Master of Arts
in the Department of
Bacteriology

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The University of British Columbia
April, 1932.

ACKNOWLEDGMENT

I wish to express my appreciation to Dr. D. C. B. Duff for his assistance in this work.

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Contents

Historical Study of Furunculosis..... I

Introduction..... 1

Examination of cultures..... 2

(a) Media..... 2
(b) Staining Methods..... 3
(c) Methods and Tests Used..... 4
(d) Morphology of the bacillus..... 7
(e) Staining qualities..... 7
(f) Cultural characteristics..... 8

Effect of pH. on the Growth and the Pigment Production....14

Viability of B. salmonicida.....15

(a) Distilled water.....15
(b) Tap water.....15
(c) Tap water plus sodium chloride.....16

Effect of Temperature on B. salmonicida.....17

Relation to Oxygen.....18

Pathogenicity of B. salmonicida.....19

(a) Methods used in pathogenicity experiments.....19
(b) Detailed description of Goldfish experiments.....21
(c) Other organisms isolated from Goldfish.....53
(d) Table illustrating Goldfish experiments.....54
(e) Experimental inoculation of rabbits with B. salmonicida.....58

Contents (continued).

Influence of the organic content of water on the organism, B. salmonicida.....61

(a) Preparation of water of varying organic content..61
(b) Chemical analysis.....62
(c) Testing the Inhibitive Action of Organic Material Upon the Growth of B. salmonicida.....65
(d) Table illustrating record of growth on plates of set number 3.....68
(e) Graph illustrating record of growth on plates of set number 3.....69

Conclusion.....70

(1) Carbohydrate reactions.....70
(2) Pigment solubility.....70
(3) Reaction of medium.....71
(4) Viability of the organism.....71
(5) Temperature.....72
(6) Relation to oxygen.....73
(7) Pathogenicity Experiments on Fish.....73
(8) Pathogenicity Experiments on Rabbits.....75
(9) Effect of organic material on the growth of the organism.....76

Summary.....78

Bibliography.....79

STUDIES ON B. SALMONICIDA.

Historical Study of Furunculosis.

As early as 1894 Emmerich and Weibel in Germany, isolated and named Bacillus or Bacterium salmonicida, the causative organism of furunculosis in the Salmonidae. Some years later Marsh in Michigan, U. S. A., described an organism which he had called Bacterium truttae, and which he stated to be pathogenic only to domesticated fish, not to those found in natural waters. This organism was later found to be identical with the B. salmonicida of Emmerich and Weibel, and their nomenclature was given preference.

In 1911 Plehn stated that until 1909 the epidemic had been seen only in domesticated waters, but after that time repeated epidemics were reported in natural waters, finally spreading throughout central Europe. According to her, old salmon were more susceptible to the disease than the young, but that a large number of deaths also occurred among the yearlings. As well as salmon and trout; pike, carp, and tench were also affected by the disease.

A. T. Masterman in his report in 1911 suggested that during that summer the high mortality among fresh water fish in the rivers of England was due in some part to the drought and the high temperatures of that year, but that in certain rivers the mortality was outstanding, both in intensity

and in length. The grayling, peal, and bream were affected as well as the salmon and the trout. These appeared to have died from some specific cause which Dr. Arkwright reported as a furunculosis epidemic, a disease, the progress of which, when at its height, was very rapid. The mortality was so rapid that in many cases no signs of the disease were observable on the exterior of the fish. From the dead fish Arkwright isolated an organism which he stated to be probably identical with the B. salmonicida of Emmerich and Weibel, and the B. truttae of Marsh. Following this epidemic Arkwright made an exhaustive study of the bacterium.

Mettam, in 1914, reported on an outbreak of furunculosis in the River Liffey in Ireland. He stated that the fish dead from the disease "show numbers of well developed boils beneath the skin and in the subcutaneous and muscular tissues" but that these symptoms may be absent in other fish killed by the same organism.

"Care and Diseases of Trout", the work of H. S. Davis in 1928, gives us a description of furunculosis and describes it as occurring "in epidemic form only among trout and salmon", though it may also affect a large number of fresh water and marine fish. Davis also remarks on the development of boils, and on the particularly heavy toll among the young trout.

Horne, in August, 1928, reported on the importance of carriers in the spread of the disease. He found these to be

the main resevoir of infection, for the organism could be isolated from them when no furuncles were present, seventy-seven days after the incidence of the disease. Plehn, in her work, had already remarked that the bacillus is met with "fairly frequently in the gut of outwardly healthy fish in infected waters".

Two years later Isobel Williamson made further reports on furunculosis of the Salmonidae showing that the epidemic had increased both in river fish and in trout farms during 1928. In 1930 she advocated the disinfection of the fish ova in the farms, stating that they are a possible vehicle of infection. With Eleanor Anderson in the same year she found B. salmonicida to be a homogeneous species and stated that it could be identified by the complement-fixation reaction which, in this case, requires a rather difficult technique described by these workers in "The Identification of B. salmonicida by the Complement-Fixation Test - A Further Contribution to the Study of Furunculosis of the Salmonidae".

STUDIES ON B. SALMONICIDA.

Introduction.

An investigation of the epidemic reported for the third year among the game fish of the Elk River near Fernie, British Columbia, was begun by Dr. D. C. B. Duff on August 12th, 1931. The area of water covered was of about forty miles, extending from below Elko to almost fifteen miles above Fernie. The deadfish, all grayling, were recovered from the river. All bore certain characteristic marks, namely a haemorrhagic, almost circular area between the pelvic fins, which often extended to the fin bases. Some specimens were found to be marked in a similar manner on the ventral surface. The peritoneum and the omentum were heavily infiltrated with blood, and in two of the fish the posterior half of the intestine was swollen and haemorrhagic. In the majority of cases the kidney was a fluid mass.

The diseased fish which were obtained were examined at Fernie immediately after their recovery from the stream. Pure cultures of a brown pigmenting organism closely resembling the B. salmonicida of Emmerich and Weibel and the B. truttae of Marsh were obtained from the kidney, the dorsal blood vessel and the inside pelvic sore. The cultures obtained by Dr. Duff were grown on nutrient agar and kept for further examination.

Examination of cultures.

Media.

Nutrient Agar - Bacto Agar 1.5%-pH. 7.0.

Blood Agar - Bacto Agar 1.5% with 5 to 10 drops freshly drawn heart blood from rabbit per plate.

Nutrient Broth - Bacto Broth - pH. 6.8.

Carbohydrate Broths - Sterile nutrient broth to which 1% of the sterilised sugar, and brom thymol blue, at the rate of 1cc. of a 1.6% alcoholic solution to the litre, as indicator, are added in an aseptic manner. pH. 7.0.

Gelatin - Bacto Nutrient Gelatin.

Litmus Milk - Fresh separated milk to which azo-litmin is added as indicator.

Nitrate Broth - Nutrient broth to which 0.1% of potassium nitrate is added.

Potato - Fresh raw potato soaked 24 hours in running water.

Tryptophane Broth - 1% solution of Bacto Tryptophane Broth.

Serum - Loeffler's Blood Serum. pH. 7.4-7.6.

Starch Media - (a) Nutrient broth to which 0.2% soluble starch is added. pH. 6.8.

(b) Nutrient agar to which 0.2% soluble starch is added. pH. 6.8.

All media were sterilised for 20 minutes with 15 pounds pressure, with the exception of milk, which was steamed for 30 minutes on three successive days.

Staining Methods.

With a Simple Aniline Dye.

Flooded with stain for one minute, washed, blotted, and examined.

Gram Stain.

Flooded with anilin gentian violet for one half minute.

Washed in water.

Flooded with Gram's iodine solution for one and one half minutes.

Washed in water.

Decolorized with 95% alcohol until no more blue colour came out.

Washed in water.

Counterstained with acqueous fuchsin one half minute.

Washed in water, blotted dry, examined.

Wright's Stain.

Flooded completely with Wright's stain, left three minutes, then added an equal volume of water, drop by drop. Blotted dry, and examined.

Methods and Tests Used:

Stock cultures of all organisms were sown on nutrient agar slants. pH. 7.0.

Cultures were examined daily, and any changes recorded.

The Preparation of Nutrient Agar of a High Alkaline pH.

It was found that on sterilisation of nutrient agar of a high alkaline pH., when the pH. was raised from pH. 7.0 to a pH. above 8.0, due to some chemical action, perhaps hydrolysis, the medium acquired a dark brown coloration. This was not suitable for the examination of the pigment producing abilities of the organism so the following method was used: The agar and the alkali were sterilised separately, thenmixed in an aseptic manner after cooling slightly. Finally this was tubed, also in an aseptic manner, slanted, and used for cultivation of the organism.

Nitrates.

Tested for reduction of nitrates to nitrites using three drops Trommsdorf's reagent (100 cc. boiling 20% zinc chloride solution to 4 grams of starch. After dissolving dilute with water, add 2 grams zinc iodide. Dilute to 1 liter and filter), then 1 drop dilute sulphuric acid (diluted 1:3). A blue colour indicates the presence of nitrites.

Indol.

Tryptophane broth. 1. Tested crude cultures using Clough's Modification of the Ehrlich test, with the following reagents:

(a) Paradimethylaminobenzaldehyde.

(b) Hydrochloric acid diluted 1:3.

(c) Chloroform U. S. P.

Method:

To 5 cc. crude culture added 0.5 cc. reagent (a) and 1 cc. reagent (b). Placed in boiling water for 20 seconds, shaking vigorously, placed in ice water ½ minute. Extracted with 1 cc. reagent (c). Compared with standards made in the same way. A pink colour indicated a positive reaction.

2. Tested the dialyzed culture using Clough's Modification of the Ehrlich test. For dialysis followed the method described in "Detection of Indol in Bacterial Cultures"- Duff, using cellophane as the dialyzing membrane.

Tested for indol after 7 days, 14 days and 21 days.

Hydrolysis of starch.

(a) Using agar plus 0.2% soluble starch. Streaked plates and incubated. Flooded the surface of the plate with a saturated solution of iodine in 50% alcohol. The clear zone outside the area of growth indicated the extent of the starch destruction.

(b) Using broth plus 0.2% soluble starch. Followed Eckford's method. Examined on the 2nd, 4th, 7th, and 10th days. Tested for acid production using brom thymol blue as indicator. Tested for hydrolysis of starch by adding one drop dilute iodine solution to one drop culture on the spot plate. A blue colour indicates no hydrolysis, a reddish brown colour partial hydrolysis with the production of erythrodextrin, clearness indicates complete hydrolysis with the production of dextrin or perhaps glucose.

Tested for the reduction of sugar with Fehling's solution by boiling 1 cc. Fehling's solution and 1 cc. solution 2 together for five minutes. Then added 2 cc. of the culture filtrate drop by drop. A yellow to orange precipitate indicates the presence of sugar.

McFarland Turbidity Standards.

Used only standards number 1, 2, and 4. These were prepared in the following manner. The turbidities were made by adding barium chloride in a 1% sulphuric acid solution forming different concentrations of barium sulphate. For number 1, 1 cc. of barium chloride solution was added to 99 cc. of water; for number 2, 2 cc. of barium chloride solution were added to 98 cc. of water; and for number 4, 4 cc. of barium chloride solution were added to 96 cc. of water. These were made up in tubes, sealed, and labelled.

Morphology of the bacterium.

The organism was found to be pleomorphic. In young agar cultures small Gram negative coccoid forms, inclining to ovoid in some cases, were discovered both singly and in masses. The average diameter of these forms was found to be 0.5 microns to 1 micron. In older cultures, especially in broth, gelatin and serum, on which the organism was later sown, rods or bacillary forms with rounded ends were observed. These were of an average length of 0.75 microns to 2 microns with a width of 0.5 microns to 0.8 microns. Spherical forms were occasionally noticed among these. No spores or capsules were observed and no true motility.

Staining qualities.

The organism stains easily with the ordinary aniline dyes. The Gram stain is negative and is often faint.

Cultural Characteristics.

For ordinary maintenance of growth nutrient agar with a pH. of 7.0 was used. For examination of the cultural characteristics the organisms used were those isolated from fresh grayling and salmon. The organisms were sown on gelatin, litmus milk, nutrient broth, nutrient agar, Eckford's broth, potato, nitrate broth, tryptophane broth, and Loeffler's blood serum, while nutrient agar and nutrient agar plus 0.2% starch were used for plating.

The cultures were examined daily for any changes in appearance and all results recorded.

Agar slants.

In 2 days - white, moderate, effuse, glistening convex growth which was translucent and slimy. A brownish pigment in the medium.

In 7 days - an abundant, effuse growth which was grayish in colour while the medium was brown throughout.

In 13 days - the medium was a dark brown throughout.

Gelatin stab.

In 2 days - filiform growth, liquefaction at the top in the form of a small sac. In some cases a sac like liquefied portion appeared at the bottom of the stab.

In 3 days - abundant growth with saccate liquefaction.

In 7 days - infundibuliform liquefaction. Sediment clings to the sides of the tubes.

In 8 days - complete liquefaction.

In 20 days - a brown ring about one centimeter in height at the top of the medium. This later extended throughout the medium.

Loeffler's blood serum.

In 1 day - a moderate, yellowish, smooth, filiform, glistening growth.

In 4 - 5 days - the growth became effuse, the medium had a brownish tinge, gas bubbles were noticed in the cultures.

In 6 days - the serum was partially digested and brown in colour. Later this became a mahogany brown in colour and all digested.

Nutrient broth.

In 2 days - an abundant growth with floccules in the tube and sediment clinging to the sides of the tube. In some cases large films of growth were observed in the centre of the tube.

In 7 days - the growth became more abundant and viscid on agitation. A slight ring of growth was noticed at the top of the culture.

In 7 - 20 days - a brown ring appeared at the top of the tube which spread gradually throughout the medium until the whole became a dark brown in colour.

Potato.

In 2 days - the growth was very scant or absent.

In 5 days - a scant, creamy, raised, filiform growth.

In 7 days - the growth remained about the same, later becoming a dirty greyish colour.

Marsh, Davis and Mettam all failed to obtain visible growth on potato, but when smears were made by Mettam after one month the organism was found on the slide.

Litmus Milk.

In 2 days - no changes.

In 7 days - the milk was coagulated but not acid.

In 13 days - the milk was coagulated and peptonized.

In 20 days - the milk became a dirty yellow colour.

Eckford's Broth.

Original pH. of broth 6.9 - 7.0.

In 2 days pH. was 6.0.

In 3 days pH. was 5.8.

In 5 days pH. was 5.6.

In 9 days pH. was 5.4.

When iodine was added to the cultures after two days the solution became clear showing complete hydrolysis of starch.

When tested with Fehling's solution for sugar reduction no reduction of the sugar could be observed even after nine days.

Nitrate broth.

When tested with Trommsdorf's reagent nitrites were observed within 5 to 7 days.

Tryptophane broth.

In 7 days - tested crude culture for indol. No indol.
In 14 days - no indol in crude culture.
In 21 days - no indol in crude culture.

When tested by the dialysis method as described by D. C. B. Duff in his work on "Detection of Indol in Bacterial Cultures" negative results were obtained after 7, 14 and 21 days.

In 21 days pigment was produced in tryptophane broth - a ring of 2 centimeters in height at the top of each culture.

Agar plates.

The colonies were very small, circular, and finely granular with entire edges. They became slightly convex and translucent. As they became older they could be split and pushed over the surface of the plate. After 3 to 5 days the medium became a dark brown in colour.

Agar plus 0.2% starch.

After 7 days iodine was poured over the plates which had been streaked. Clear haloes appeared around the streaks showing that the organism to utilized starch moderately. A slower growth than that observed on nutrient agar plates was noticeable on these plates.

Blood agar plates.

In 2 days - areas of growth appeared with clear, translucent areas around them.

In 3 days - the colonies were round, smooth, raised, entire and finely granular. Clear areas around all colonies.

In 5 days - the plates became a dark brown, the growth a dirty, greyish colour.

In 7 days - the growth became greenish.

Odour.

No odour was noticeable on any of these cultures.

Carbohydrate reactions.

The organisms were sown on the following carbohydrates and the changes recorded as below:

glucose (dextrose)	+ in 2 days.
lactose	- in 2 - 3 days.
saccharose (sucrose)	- in 1 - 2 days.
laevulose	+ in 2 days.
mannite	+ in 2 - 3 days.
salicin	+ in 2 - 3 days.
galactose	+ in 2 days.
raffinose	- in 2 - 3 days.

inulin	- in 2 - 3 days.
maltose	+ in 2 - 3 days.
arabinose	+ in 1 day.
xylose	+ in 3 days.

Gas was produced in glucose and mannite tubes, but was often very slight.

Pigment Solubility.

The pigment produced by the organism was found to be soluble in water, and when ethyl alcohol was added to an agar slope the pigment diffused out into the alcohol.

Effect of pH. on the Growth and the Pigment Production.

Cultures of B. salmonicida were sown on nutrient agar slants of varying pH. to test for the range of growth and of pigment producing ability of the organism.

Alkaline Media.

Agar of pH. 9.0 - moderate growth with pigment production confined mainly to the growth area. Pigment was produced after seven days.

Agar of pH. 9.2 - scant growth with no pigment production.

Agar of pH. 9.4 - no growth.

Acid Media.

Agar of pH. 5.4 - moderate growth with moderate pigment production in five days.

Agar of pH. 5.2 - scant growth with no pigment production.

Agar of pH. 5.0 - very scant growth with no pigment production.

Agar of pH. 4.8 - no growth.

There appeared to be a greater amount of pigment produced in a shorter time on a slightly alkaline medium than on a slightly acid medium. The optimum pH. for pigment production was between 7.4 and 7.8

Viability of B. salmonicida.

(a) Distilled water.

In distilled water the organism was not often recovered after 24 hours, but in some cases could be recovered after 3 days.

The method used was the following:- 250 cc. sterile distilled water were placed in a flask and inoculated with 1 cc. of a number one McFarland suspension of the organism. Before plating each flask was thoroughly shaken. Plates were made every twenty-four hours, and in duplicate in all cases. Results were recorded as below.

Sample A -	made	27/11/31	- none	recovered	30/11/31.
Sample B -	"	27/11/31	- "	"	30/11/31.
Sample C -	"	4/12/31	- "	"	5/12/31.
Sample D -	"	4/12/31	- "	"	7/12/31.
Sample E -	"	23/2/32	- "	"	24/2/32.
Sample F -	"	23/2/32	- "	"	24/2/32.
Sample G -	"	1/3/32	- "	"	4/3/32.
Sample H -	"	1/3/32	- "	"	3/3/32.
Sample I -	"	15/3/32	- "	"	19/3/32.
Sample J -	"	15/3/32	- "	"	19/3/32.

(b) Tap water.

Sterile tap water cultures were made in the same manner, and tested daily for the appearance of growth on nutrient agar plates.

Sample A -	made	2/12/31	- none	recovered	5/12/31.
Sample B -	"	2/12/31	- "	"	5/12/31.
Sample C -	"	7/12/31	- "	"	10/12/31.
Sample D -	"	7/12/31	- "	"	10/12/31.
Sample E -	"	23/2/32	- "	"	25/2/32.
Sample F -	"	23/2/32	- "	"	25/2/32.
Sample G -	"	1/3/32	- "	"	13/3/32.
Sample H -	"	1/3/32	- "	"	13/3/32.
Sample I -	"	15/3/32	- "	"	22/3/32.
Sample J -	"	15/3/32	- "	"	22/3/32.
Sample K -	"	15/3/32	- "	"	26/3/32.
Sample L -	"	15/3/32	- "	"	26/3/32.

(c) Tap water plus sodium chloride.

Then the viability of the organism was tested on tap water plus varying amounts of sodium chloride. Each flask contained 250 cc. sterile tap water plus a certain percentage of sodium chloride. As in the above cases one cubic centimeter of a number one McFarland suspension was inoculated into each flask. Controls of sterile tap water were made at the same time, and in the same manner. Flasks were prepared in duplicate in all cases.

Sample Number	% NaCl.	Date made		
A.	1.25	2/12/31	none recovered	8/12/31.
	.85	2/12/31	"	9/12/31.
	.5	2/12/31	"	22/12/31.
	0.0	2/12/31	"	5/12/31.
B.	1.0	23/2/32	"	3/3/32.
	.85	23/2/32	"	5/3/32.
	.5	23/2/32	"	16/3/32.
	0.0	23/2/32	"	25/2/32.
C.	3.0	29/2/32	"	9/3/32.
	1.0	29/2/32	"	11/3/32.
	.85	29/2/32	"	14/3/32.
	.5	29/2/32	"	24/3/32.
	0.0	29/2/32	"	11/3/32.

Effect of Temperature on B. salmonicida.

Sowed four nutrient broth cultures with one loopful of the organism from a nutrient agar slant and kept samples at 37°C, 28°C, 14°C (approximately) and at room temperature. Tested daily by sub cultures on nutrient broth.

Sample A.

Sowed 2/12/31 - 37°C - no growth on subcultures of 18/12/31.
28°C - growth on subcultures of 30/3/32.
14°C - growth on subcultures of 30/3/32.
room - growth on subcultures of 30/3/32.

Sample B.

Sowed 6/1/32 - 37°C - no growth on subcultures of 26/3/32.
28°C - growth on subcultures of 30/3/32.
14°C - growth on subcultures of 30/3/32.

Sample C.

Sowed 18/2/32 - 37°C - growth on subcultures of 30/3/32.
28°C - growth on subcultures of 30/3/32.
14°C - growth on subcultures of 30/3/32.

Relation to oxygen.

No work was done in regard to the growth of the organism anaerobically. It was observed that when cultures were sealed with paraffin immediately after inoculation growth was moderate but no pigment was produced even after fourteen days. When the paraffin was removed or when a sub-culture was sown on fresh nutrient agar pigment was produced in two days.

Pathogenicity of B. salmonicida.

Healthy living trout did not survive under laboratory conditions. As a result of this the following experiments were performed using goldfish as the host. The goldfish numbered 1 to 31 inclusive.

Methods used in Pathogenicity experiments.

Inoculation of fish.

Cultures on agar obtained from other fish, three to four days old, were washed off with a sterile, normal saline solution and pooled. These were then adjusted, with the use of saline solutions, to the degree of turbidity required according to the McFarland standards. The fish were held in the palm of the hand during inoculation, and a sterile syringe was used. Varying quantities of the suspension were used, and the fish were inoculated intramuscularly and intraperitoneally.

Autopsy of the fish.

For the autopsy the instruments were sterilised by boiling. A small wooden board was washed thoroughly with 95% alcohol or 0.5% phenol, and the specimen to be examined, rinsed off with tap water, then wiped with absorbent cotton soaked in 0.5% phenol. The scales were removed with sterile forceps in order to examine the exterior of the fish for the presence of haemorrhagic areas. The fish was then placed, abdomen up, on the board, fastened in position by means of pins placed in the

head and the tail, and the abdominal wall cut, beginning at the anal end. The skin was removed, pinned to the board and the visceral contents were examined. Cultures were taken from the organs with a sterile loop, sown on nutrient agar, and incubated. After two to three days plates were streaked from these cultures and incubated. Colonies resembling those of B. salmonicida were picked, sown on nutrient agar and examined for pigment production. These were then examined further for carbohydrate action and their growth on gelatin, and on litmus milk.

Detailed description of Goldfish experiments.

Goldfish number 1.

30/10/31 - inoculated intramuscularly with 2/10 cubic centimeters number 1 McFarland suspension of B. salmonicida. Left at room temperature.

31/10/31 - fish remained quiet in water, white at site of inoculation, swelling below inoculation site.

2/11/31 - swelling below inoculation site was larger.

3/11/31 - fish found dead at 9 A. M. Found at bottom of bowl, erect with tail touching the bottom.

Autopsy.

Exterior examination.

The inoculation site was covered with a soft, slimy, easily removed mass. Under low power this appeared to be dead tissue. Fish appeared bloated and pulpy around the muscles near the inoculation site.

Interior examination.

Cut a square opening around the inoculation site. Found a large abscess filled with white purulent matter. The abscess, which was four millimetres deep at the deepest point and one centimetre in diameter, was surrounded by a large, haemorrhagic area of 1¼ centimetres in diameter. The muscles around this area appeared congested. The lower portion of the fish, and also the gills appeared normal.

Cut abdomen of fish. The gall bladder was very large, and pin point black dots covered the caeca and the

intestine. The intestine did not appear abnormally congested. The liver was soft, pulpy, and almost colourless, while the kidney was covered with white spots. The swim bladder appeared normal.

Sliced fish transversely. Found a distinct red spot below the abscess, on the dorsal region. Occasional red spots were observed in the muscle area.

Sowed cultures from the liver, the kidney, the guts, the depth of the abscess and from the outside of the abscess. Examined slides taken from the white, pulpy mass outside the sore, the liver, and the depth of the abscess. Stained these with methylene blue, Gram stain, and Wright's stain.

Results of microscopic examination of slides.

1. Outside sore.

(a) Methylene blue - the slide contained in the majority small rods with rounded ends, single and in pairs, 0.5 to 0.7 microns in length and 0.3 microns in width. Some of these were almost coccus like in shape.

(b) Gram stain - The rods appeared faintly Gram negative.

(c) Wright's stain - no change in the appearance of the rods. The slides also contained tissue cells.

2. Liver.

(a) Methylene blue - the smear contained a very few short rods of from 0.5 to 1 micron in length by 0.2 - 0.5 microns in width.

(b) Gram stain - the rods appeared to be Gram negative.

(c) Wright's stain - no change in the appearance of the rods.

3. Depth of abscess.

(a) Methylene blue - an almost pure smear of short rods of 0.5 - 1 microns by .2 - 0.5 microns in length.

(b) Gram stain - the rods appeared Gram negative.

(c) Wright's stain - no change in the appearance of the rods. With Wright's stain in all cases clumps of cell appeared to be present which were thought to be due to the degeneration of cellular elements. There were few perfect cells. Some cell like bodies were also faintly visible in the methylene blue smears. These appeared egg like in shape with a dark blue stained nucleus.

The fact that the rods inside the body of the fish were larger than those from the outside seems to indicate that the abscess did not open to the outside.

Pure cultures of the organism were isolated from the abscess, the liver and the guts.

Goldfish number 2.

30/10/31 - inoculated intramuscularly with 2/10 cc. number one McFarland suspension of B. salmonicida. Left at room temperature.

31/10/31 - white material covered inoculation site.

2/11/31 - swelling at inoculation site, white material around gill opening.

3/11/31 - fish remained inactive near the bottom of the bowl. The inoculation site was swollen and discolored.

4/11/31 - fish had difficulty in retaining balance.

4/11/31 - swelling at inoculation site larger, discoloration a darker red. The wound was covered with a white fungus like growth. The fish had difficulty in retaining its balance and the exterior was completely covered with whitish material.

7/11/31 - the fish was more active, the swelling less red and less white material covered the fish. The white material on microscopic examination appeared to be clumps of cell and tissue, with few perfect cells present.

8/11/31 - at times the fish appeared almost dead.

9/11/31 - the wound appeared longer and darker underneath. A dark spot appeared on the side opposite the wound, the fish appeared fairly active.

10/11/31- a darker red line appeared around the wound, less white appeared on the wound.

12/11/31- the sore was open, all white matter had disappeared except around the edges of the sore, black matter covered the sore. Black spots appeared on the fins, the tail, the gill, and on the sides.

13/11/31- the open sore was one centimeter in diameter with black around the edges, around the gill slits, and on the sides.

14/11/31 - the sore was larger. The fish had periods of extreme activity, then inactivity.

18/11/31 - fish appeared more active.

19/11/31 - all black marks had disappeared.

23/11/31 - killed fish after 26 days. Performed autopsy.

Autopsy.

Exterior examination.

Fish appeared active, all black spots had disappeared. The sore was beginning to close.

Interior examination.

Cut a square opening around the inoculation site. Found a shallow, soft wound containing reddish matter. The flesh around the wound appeared firm and white. The gills appeared normal.

Cut abdomen. The interior organs appeared normal with the exception of the liver which was soft and pulpy.

Sowed cultures from the open wound, the liver, the heart, the kidney, the gall bladder and the dorsal blood vessel.

Made smears from the outer surface, the open wound, the spleen, inside the wound, the heart, and the gall bladder. Stained with methylene blue and examined.

Results of microscopic examination of slides.

1. Outside wound - only one or two coccus like organisms and one or two very small rods with rounded ends of .45 - .65 microns by .25 - 0.3 microns.

2. Liver - a very few short rods with rounded ends, all about the same size, of 0.7 microns by 0.2 microns. Some cellular substances were also present.

3. Heart - small rods with rounded ends of 0.5 - 1 microns by 0.2 - 0.3 microns. Also some cocci in masses and some cellular material.

4. Inside wound - a few small rods of 0.5 - 1 microns by .25 - 0.3 microns. Also observed the presence of some cellular material.

5. Spleen - a very few small rods of 0.5 - 0.8 microns by 0.2 - 0.3 microns.

6. Gall bladder - contained small rods of 0.5 - 1 microns by 0.2 - 0.3 microns.

Isolated pure cultures of B. salmonicida from the inside of the sore, the liver, the heart, and the kidney.

Apparently the fish had been able to resist the disease. If such were the case then a fish which had been scarified would probably not suffer any ill effects.

Goldfish number 3.

30/10/31 - scarified fish and rubbed culture of organism into sore.

31/10/31 - observed a small, white spot on the sore. No other changes were observed. The fish remained in apparent good health.

2/12/31 - inoculated fish intramuscularly with 2/10 cc.

number 1 McFarland suspension of B. salmonicida.

3/12/31 - fed fish with suspension of organism mixed with fish food. The faeces was long and in a thin straight line. The inoculation site was covered with white material.

4/12/31 - observed a dark, red area around the inoculation site, and a nervous twitching of the dorsal fin.

5/12/31 - fish found dead at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

No white matter on the outside of sore but a dark red line was observed below it.

Interior examination.

The inside of the sore was dark red and filled with purulent matter. The muscle area around the sore soft, but in other parts of the body was firm and white. Found a large blood clot underneath the abscessed spot. The internal organs of the fish appeared normal.

Sowed cultures on nutrient agar from the outside of the abscess, the inside of the abscess, the liver, the spleen, the kidney, the dorsal blood vessel, and the heart.

Made smears from the depth of the abscess, the liver, and the spleen. Stained with Gram stain and examined.

Results of microscopic examination of slides.

1) The depth of the abscess - the slide contained very small Gram negative rods of 0.45 - 0.8 microns in length and

0.2 - 0.3 microns in width, also Gram negative cocci of approximately 0.6 microns in diameter.

2) The Liver - the slide contained some large Gram positive rods and some Gram negative rods of 0.5 - 1 microns in length and 0.2 - 0.3 microns in width.

3) The spleen - the slide contained Gram negative rods of .45 - 0.9 microns in length and 0.2 - 0.3 microns in width, also numerous Gram negative cocci of 0.6 microns in diameter.

Isolated pure cultures of B. salmonicida from the heart, the spleen, and the liver.

Goldfish number 4.

30/10/31 - scarified fish and rubbed culture of the organism into the sore.

31/10/31 - soft white fungus like material was observed on the scarified spot.

No other changes were observed in the fish. The fish remained in good health and acted in a normal manner.

2/12/31 - inoculated 2/10 cc. number 1 McFarland suspension of B. salmonicida intramuscularly.

3/12/31 - white fungus like material was observed at the inoculation site. Added a broth culture of the organism to the fish food.

4/12/31 - The inoculation site was dark red.

5/12/31 - The inoculation site was swollen and discolored

but the fish remained active. Mixed broth culture of the organism with the fish food.

7/12/31 - the fish remained active but the wound was swollen and discoloured. The faeces was two and one half inches long, was thin and white.

8/12/31 - observed a large red haemorrhagic area around the sore. The fish remained near the top of the bowl, had convulsive movements with later periods of inactivity.

9/12/31 - the red area surrounding the sore was larger. The fish remained near the surface of the bowl.

10/12/31 - observed a large open sore. The fish remained less active.

11/12/31 - at 9 A. M. found the fish floating on its side in the bowl. Performed autopsy.

Autopsy.

Exterior examination.

A large hole, 1 centimeter in diameter and 4.5 millimetres in depth, was observed at the site of inoculation. The sore was surrounded by white, decolorised scales. The tissue in the hole was hard, the scales soft and easily removed.

Interior examination.

Cut abdomen of fish, removed skin. Connected with the open wound was a large abscessed area of 1 centimeter in diameter. This was filled with white purulent matter, and

the area surrounding the abscess was soft. A red haemorrhagic area was found on the muscle below the sore. The liver and the kidney were soft and pulpy, and whitish in colour. The swim bladder, the heart, and the spleen were all apparently normal.

Sowed cultures from the depth of the sore, the sore from the outside, the liquid in the abscess, the depth of the abscess, the clot under the abscess, the heart, the dorsal blood vessel, the spleen, the liver, the kidney, and the liquid in the dorsal area.

Made smears from the outside of the sore and the depth of the abscess. Stained with Gram stain and examined.

Results of microscopic examination of slides.

1) Outside of the sore - observed numerous small Gram negative rods with rounded ends and length of .45 - 0.9 microns with a width of 0.2 - 0.3 microns.
2) Depth of the abscess - an almost pure smear of small Gram negative rods of 0.5 microns in length and 0.2 - 0.3 microns in width.

Isolated pure cultures of B. salmonicida from the heart, the spleen, the liver, the dorsal blood vessel, the depth of the abscess, and the clot under the abscess.

These cultures did not produce any pigment on agar until subcultured four times, with a period of one week between each two subcultures.

Goldfish number 5 and number 6.

30/10/31 - added 15 cc. three day old broth culture of

B. salmonicida to the water.

No apparent effects were observed. The fish remained in good health.

2/12/31 - inoculated 2/10 cc. number 1 McFarland suspension intramuscularly.

3/12/31 - observed white matter around the inoculation site. The faeces was long, thin and colourless.

4/12/31 - a dark red discoloration was noticeable around the inoculation site.

5/12/31 - the tissue was swollen around the sore but the fish remained active.

7/12/31 - a red haemorrhagic area was observed around the sore, white matter was given off.

9/12/31 - the fish remained near the surface of the water.

12/12/31 - the fish grew more active until

29/12/31 - when it was placed in the incubater at 26°C.

16/1/32 - inoculated again with 3/10 cc. number 1 McFarland suspension of B. salmonicida.

18/1/32 - noticed a slight swelling around the inoculation site . No other effects were observed.

2/2/32 - death of goldfish No. 6 due to accident. No cultures were obtained.

9/2/32 - Removed goldfish No. 5 to room temperature.

Goldfish number 7.

2/12/31 - inoculated intramuscularly with 2/10 cc. number 1 McFarland suspension of B. salmonicida.

4/12/31 - red haemorrhagic area surrounded the inoculation site.

5/12/31 - the tissue around the inoculation site was swollen and red. The fish remained active.

16/1/32 - the fish was apparently recovering from its previous inoculation. Reinoculated intramuscularly with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Placed in incubator at 28°C.

17/1/32 - found fish dead at 3 P. M. Performed autopsy.

Autopsy of fish number 7.

Exterior examination.

(a) left side - The pectoral fin was turned anteriorally. At the base of the pectoral fin was a haemorrhagic area of 2 by 4 millimetres. At the base of the caudal fin was a haemorrhagic area 2 mm. square. The anus appeared normal.

(b) right side. - The pectoral fin was normal. The site of injection was not haemorrhagic but there was a marked haemorrhagic area above the caudal fin. On the anterior belly was a suffused area of 1 cm. by 3 mm. between the pectoral fins.

Interior examination.

There were numerous dark spots of ½ millimeter in diameter on the walls of the intestine and the peritoneum. The swim bladder was intact, the liver soft, and the upper intestine filled with gas. The deep muscle layers contained

no haemorrhagic areas but when the scales were scraped off many small haemorrhagic areas were observed all along the flank.

Sowed cultures on nutrient agar from the liver, the body cavity, and the heart. Incubated at 26°C.

Isolated pure cultures of B. salmonicida from all of these.

Goldfish No. 8.

2/12/31 - inoculated intramuscularly with 2/10 cc. number 1 McFarland suspension of B. salmonicida.

From 3/12/31 to 29/12/31 - the symptoms were the same as those observed in goldfish number 5.

16/1/32 - inoculated intramuscularly with 3/10 cc. number 1 McFarland suspension of B. salmonicida.
Incubated at 26°C.

17/1/32 - fish found dead at 3 P. M. Autopsy performed.

Autopsy of fish.

Exterior examination.

a) right side - appeared normal.

b) left side - The pectoral was turned anteriorally and had a haemorrhagic area at the base. Found a haemorrhagic area at the base of the operculum. Removed scales. There was no visible haemorrhagic area with the exception of one soft spot on the under side just below the dorsal fin. The contents of the body cavity were soft and contained much

fluid. The swim bladder was intact, the intestine constricted in spots, and full of gas. The liver was almost fluid. The deep muscle was haemorrhagic in spots.

Sowed cultures on nutrient agar from the peritoneal fluid, the heart, and the kidney. Incubated at 26°C.

Isolated pure cultures of B. salmonicida from all of these.

Goldfish number 9 and number 10.

4/1/32 - inoculated 2/10 cc. number 1 McFarland suspension of B. salmonicida both intraperitoneally and intramuscularly. Incubated at 26°C.

5/1/32 - found both fish floating on side in bowl at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

Under the pectoral fins were deep, red, soft areas. The whole of each fish was covered with soft, white matter. About ½ inch from the tail there was a dark, red, bloody area which did not go to any depth.

Interior examination.

All the organs of the fish appeared normal.

Sowed cultures of Goldfish number 9 from the heart, the intestinal contents, the pectoral fin base, and the haemorrhage near the tail.

Sowed cultures of Goldfish number 10 from the blood, the dorsal blood vessel, the red in the muscle area, the liver,

the inside of the sore under the pectoral fins, and the vent.

Isolated pure cultures of B. salmonicida from all of these cultures.

Goldfish No. 11.

4/1/32 - inoculated with 2/10 cc. number 1 McFarland suspension of B. salmonicida both intraperitoneally and intramuscularly.

6/1/32 - observed reddish spot at the inoculation site.

7/1/32 - found fish floating on side at 9 A. M.

Performed autopsy.

Autopsy of fish.

Exterior examination.

Observed a large dark patch under the scales with an abscess farther down opposite the pelvic fin, about 1 centimetre from the inoculation site. This was soft, dark, red and filled with soft, red matter. The fish itself was very soft, the skin broke easily, and the interior appeared to be in a soft decayed state.

Interior examination.

The intestine appeared slightly congested, the spleen was very soft and fell to pieces easily. The body was haemorrhagic all through the muscle area, even on the side opposite the inoculation site.

Sowed cultures on nutrient agar from the abscess before searing, the seared abscess, the intestinal contents,

the muscle opposite the inoculation site, the spleen, the fluid near the back.

Isolated pure cultures of B. salmonicida from all of these.

Goldfish number 12.

4/1/32 - inoculated with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Left at 26°C.

6/1/32 - a small red spot at the inoculation site.

7/1/32 - found dead at 9:30 A. M. erect in bottom of jar.

Performed autopsy.

Autopsy of fish.

Exterior examination.

No definite abscess at inoculation point on the shoulder but the flesh was soft and haemorrhagic. Several black areas on both sides. The bases of the pectorals appeared haemorrhagic.

Interior examination.

The gall bladder was almost as large as the liver, the hind gut congested and swollen. Small haemorrhagic areas were observed about the anus.

Sowed cultures on nutrient agar from the muscle, the inoculation site, the peritoneal fluid, the mid gut, the liver, the bile from the gall bladder, the kidney, the dorsal blood vessel, the soft muscle patch, and from a dorsal deep muscle block. Incubated at 26°C.

Did not obtain cultures of B. salmonicida from the kidney, the dorsal blood vessel, and the dorsal deep muscle block. Isolated pure cultures from all the rest.

Goldfish number 13.

9/1/32 - inoculated fish with 2/10 cc. number 1 McFarland suspension of B. salmonicida intramuscularly. Left at 26°C.

16/1/32- the inoculation of 9/1/32 did not seem to have affected the fish in any way. Reinoculated intraperitoneally with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Left at 26°C.

17/1/32- Fish found dead at 3:30 P. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

(a) Right side - the pectoral fin was turned out, and had a small haemorrhagic area at the base. A haemorrhagic area on the peduncle showed through the scales as did a congestion at the inoculation site. When the scales were removed many minutes haemorrhagic spots were observed over the entire side.

(b) Left side - The left pectoral fin was normal. When the scales were removed many minutes haemorrhagic spots were observed over the entire side.

Interior examination.

The body walls were soft and rotten. There was

much fluid in the body cavity. The swim bladder was intact. Dark spots were observed on the peritoneum. Several definite soft haemorrhagic areas were observed below the skin. These were of 2 mm. in diameter with a pin point of pus in the centre of each.

Sowed only one culture and this from the peritoneum on nutrient agar. Incubated at 26°C.

Obtained a pure culture of B. salmonicida from the peritoneum.

Goldfish number 14.

9/1/32 - inoculated intramuscularly with 2/10 cc. number 1 McFarland suspension of B. salmonicida.

16/1/32 - the inoculation of 9/1/32 did not seem to have affected the fish in any way. Reinoculated intraperitoneally with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Left at 26°C. Found fish dead at 3 P. M. Death was due to injury to the swim bladder. No cultures of B. salmonicida were obtained.

Goldfish number 15.

9/1/32 - inoculated fish with 2/10 cc. number 1 McFarland suspension of B. salmonicida. Incubated at 26°C.

16/1/32 - reinoculated with 3/10 cc. number 1 McFarland suspension of B. salmonicida intraperitoneally. Incubated at 26°C.

17/1/32 - found dead at 3:30 P. M. Performed autopsy.

Autopsy.

Exterior examination.

(a) Left side - pectoral fin turned out with haemorrhagic area of 5 mm. by 3 mm. at the base. Haemorrhagic spot at inoculation site.

(b) Right side - pectoral fin turned out with haemorrhagic area of 5 mm. by 2.5 mm. at the base.

After removal of scales, both sides showed small haemorrhagic spots.

Interior examination.

The flesh was too soft to observe. The swim bladder was intact.

Sowed one culture only, and this from the peritoneum. Incubated at 26°C. Finally isolated a pure culture of B. salmonicida from this.

Goldfish number 16.

16/1/32 - inoculated intraperitoneally with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Incubated at 26°C.

17/1/32 - fish found dead at 3:30 P. M. Performed autopsy.

Autopsy.

Exterior examination.

(a) Right side - the pectoral fin was turned downward, and had a slight haemorrhagic area at base. Removed

scales, found only one or two haemorrhagic spots on the belly.

(b) Left side - Observed a haemorrhagic area the dorsal side of the operculum. Otherwise the left side appeared normal. Removed scales, no haemorrhagic spots were observed.

Interior examination.

There were no visible spots or haemorrhages in the muscle. The visceral organs appeared intact. The belly was punctured at the site of inoculation.

Obtained only one pure culture of B. salmonicida. This was from the kidney.

Goldfish number 17.

16/1/32 - inoculated fish intraperitoneally with 3/10 cc. number 1 McFarland suspension of B. salmonicida. Left at 26°C.

18/1/32 - fish found dead at 9 A. M. Performed autopsy.

Autopsy.

Exterior examination.

(a) Right side - The pectoral fin was turned anteriorally. The whole body of the fish was dark, the anus reddish, and the left fin turned out. A large haemorrhagic area of 2 - 3 mm. in diameter was observed just below the pectoral fin. Below this was a large dark area almost ½ inch in diameter. Purulent matter was exuding from the haemorrhagic area.

(b) Left side - suffused haemorrhagic areas were observed on the left side.

Interior examination.

Dark spots were found on the intestinal wall and on the peritoneum. The swim bladder was intact, the intestine congested. The lower half of the fish contained no haemorrhages.

Sowed cultures from the liver, the haemorrhage, the seared abscess, the dorsal blood vessel, and the peritoneal fluid.

Isolated pure cultures of B. salmonicida from all of these cultures.

Goldfish number 18.

21/1/32 - inoculated fish intraperitoneally with 3/10 cc. number 1 McFarland suspension from cultures from the kidney and the peritoneal fluid of fish No. 8. Incubated at 26°C.

22/1/32 - found fish floating in water at 9 A. M. Performed an autopsy.

Autopsy of fish.

Exterior examination.

The fish appeared normal.

Interior examination.

The liver appeared very soft and covered with small dark spots. Otherwise the organs appeared in a normal condition.

Sowed cultures on nutrient agar from the liver spots, the liver, the heart, the spleen, and the peritoneal fluid. Incubated at 26°C.

Isolated pure culture of B. salmonicida from the heart, the liver, the liver spots and the peritoneal fluid.

Goldfish number 19.

21/1/32 - inoculated the fish intraperitoneally with 3/10 cc. number 1 McFarland suspension of culture of B. salmonicida isolated from fish number 8. Left at 26°C.

22/1/32 - found floating at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

There was no exterior evidence of the disease.

Interior examination.

The liver was very soft and fluid, and covered with small spots. All other organs appeared to be in a normal condition.

Sowed cultures on nutrient agar from the liver, the bile, the heart, the peritoneal fluid, the spots on the liver. Incubated at 26°C.

Isolated pure cultures of B. salmonicida from the heart, the liver, the bile, the peritoneal fluid, and the spots on the liver.

Goldfish number 20.

21/1/32 - inoculated the fish intraperitoneally with 3/10 cc. number 1 McFarland suspension of culture of

B. salmonicida isolated from fish number 8. Left at room temperature.

22/1/32 - found floating at top of bowl at 12 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

A large open sore surrounded by a large haemorrhagic area was present at the inoculation site. This had bloody matter exuding from it for one hour before death of the fish. The muscle area surrounding the anus, and that under the pectoral fins were all haemorrhagic. A lesion had formed under the caudal fin, and the muscle between the pelvic fins was also haemorrhagic.

Interior examination.

The intestine was very haemorrhagic all the way from the anus to the stomach. The ovaries were large. All organs were haemorrhagic in patches. The swim bladder was unaffected.

On cross sectioning a line of haemorrhage was found which extended all along the back of the fish. Small haemorrhagic spots were observed deep in the muscle and surrounding the back bone. These existed even in the head area.

Sowed cultures on nutrient agar from the peritoneum, the posterior intestinal contents, the bile, and the kidney. Isolated pure cultures of B. salmonicida from all these organs.

Goldfish number 21.

21/1/32 - inoculated fish intraperitoneally with 3/10 cc. number 1 McFarland suspension B. salmonicida obtained from Goldfish number 8. Left at room temperature.

22/1/32 - found floating at top of bowl at 1 P. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

The fish was very bloated in appearance with a haemorrhage of 0.5 cm. in width from the site of inoculation up the side for 3 cm. The pectoral fins and the anus were also haemorrhagic and a small haemorrhagic spot was found directly across from the site of inoculation.

Interior examination.

The intestinal organs were haemorrhagic and the posterior end of the intestine was swollen to a distance of 2 cm. from the anus. Blood clots were numerous in the omentum.

On cross sectioning small haemorrhagic spots were found around the back bone, all along the lateral line and throughout all the muscle area.

Cultures were sown on nutrient agar from the peritoneum, the heart, the kidney, the intestinal contents, and from one of the blood clots.

Pure cultures of B. salmonicida were isolated from the heart, the peritoneum, the kidney, the intestinal contents. Both Goldfish number 20 and Goldfish number 21 remained very near the surface of the water before death.

Goldfish number 22.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of a 1:100,000 dilution of a number 4 McFarland suspension of B. salmonicida. Left at 26°C.

1/2/32 - no visible changes were observed. On this date fish died as the result of an accident. No cultures were obtained.

Goldfish number 23.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of 1:100,000 dilution of a number 4 McFarland suspension of B. salmonicida. Left at 26°C.

9/2/32 - no changes were visible so removed to room temperature.

20/3/32 - Fish was active, and unaffected by the inoculation.

Goldfish number 24.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of a 1:100 dilution of a number 4 McFarland suspension of B. salmonicida. Left at 26°C.

9/2/32 - no changes had been observed so removed to room temperature.

24/2/32 - black spots were observed on the sides of the fish and on the tail.

29/2/32 - the fish had a tendency to lose its balance. The spots were becoming larger, and existed around the pelvic fins and gill openings. The fish remained motionless near the top of the water .

2/3/32 - the fish remained in the same inactive state. Killed at 12 A. M. Performed autopsy of fish.

Autopsy of fish.

Exterior examination.

Small haemorrhagic spots were observed near the pelvic fins and along the muscle area after the removal of the scales.

Interior examination.

All the organs appeared normal. The intestine was haemorrhagic near the anus.

Sowed cultures on nutrient agar from the heart, the dorsal blood vessel, the kidney, the liver, the anterior portion of the intestine, the posterior portion of the intestine, and the spleen.

Isolated a pure culture of B. salmonicida from the posterior portion of the intestine only.

Goldfish number 25.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of a 1:100 dilution of a number 4 McFarland suspension

of B. salmonicida. Left at 26°C.

1/2/32 - the fish remained active. A haemorrhage appeared near the pectoral fin on the right side.

3/2/32 - death due to accident. No cultures obtained.

Goldfish number 26.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of a 1:100 dilution of a number 4 McFarland suspension of B. salmonicida. Left at room temperature.

27/1/32 - no external changes were visible but the fish was inactive and remained near the surface of the water.

2/2/32 - fish found dead at 1 P. M. Performed autopsy.

Autopsy of fish.

Exterior symptoms.

The only visible sign was a small hole at the site of inoculation.

Interior examination.

The anus was red at the opening, the intestine haemorrhagic, and small haemorrhages were observed in the muscle layers. Otherwise the organs appeared unaffected.

Sowed cultures on nutrient agar from the heart, the intestinal contents, the bile, the fluid at back and peritoneal fluid.

Isolated pure cultures of B. salmonicida from the heart, the intestinal contents, the bile, the fluid at the

back, and the peritoneal fluid.

Goldfish number 27.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. of a 1:100 dilution of a number 4 McFarland suspension of B. salmonicida. Left at room temperature.

27/1/32 - the fish remained inactive.

2/2/32 - the fish remained near the surface of the water.

3/2/32 - the fish found dead at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

At the inoculation site was a large hole with a soft area surrounding it. There were red haemorrhagic spots under the scales near the tail, the base of the anal fin was haemorrhagic, and on pressure red matter exuded from the anal opening. A large dark patch was observed on the side opposite the inoculation site.

Interior examination.

The intestine was congested and haemorrhagic to one eighth of an inch from the anal opening. Otherwise the visceral organs appeared normal. The muscle area was haemorrhagic throughout.

Sowed cultures on nutrient agar slants from the peritoneum and the heart.

Used fish for sectioning. Haemhorragic areas were found from the tail to the head and surrounding the backbone.

Goldfish number 28.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. number 4 McFarland suspension of culture of B. salmonicida. Incubated at 26°C.

28/1/32 - the fish was floating at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

The anus was dark red and surrounded by a haemorrhagic area, small red spots appeared around the mouth, and dark patches were visible through the skin.

Interior examination.

The haemorrhage at the anus was exuding a dark red liquid, a haemorrhage was observed on the right side near the pectoral fin. This was of a diameter of 0.5 centimetres. The interior of the fish was inflated and congested, blood clots were found in the peritoneum. On slicing haemorrhages were found throughout the muscle layers, and down the lateral line.

Sowed cultures on nutrient agar from the intestinal contents, the back fluid, haemorrhage along the lateral line, the bile from the gall bladder, the anus, the haemorrhage at the tail, the haemorrhage on the right side, the peritoneal fluid, clot in the peritoneum, and the heart.

Obtained cultures of B. salmonicida from all of these cultures.

Goldfish number 29.

26/1/32 - inoculated fish intraperitoneally with 2/10 cc. number 4 McFarland suspension of culture of B. salmonicida. Incubated at 26°C.

28/1/32 - the fish was found dead at the bottom of the bowl on its side at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

At the inoculation site was a hole with a small haemorrhage, 4 cm. in diameter. Red matter exuded from the anus.

Interior examination.

The intestine was haemorrhagic and distended. Clots were found in the peritoneum. The other organs appeared normal.

Sowed cultures on nutrient agar from the intestinal contents, clot in the peritoneum, the peritoneal fluid, the heart, the anus, and the haemorrhage at the inoculation site.

Cultures of B. salmonicida were not obtained from the clot in the peritoneum or from the anus.

Goldfish number 30.

26/1/32 - mixed 10 cc. of a number 4 McFarland suspension of B. salmonicida with the fish food. Left at room temperature.

1/2/32 - inoculated the fish intraperitoneally with 2/10 cc.

number 4 McFarland suspension of B. salmonicida. Left at room temperature.

2/2/32 - found fish floating at 9 A. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

There were no exterior symptoms, with the exception of a small hole under the scales at the inoculation site. This was surrounded with soft tissue.

Interior examination.

The intestine was haemorrhagic. Otherwise the organs appeared normal. A blood clot was observed in the peritoneum.

Sowed cultures on nutrient agar from the heart, the peritoneal fluid, the fluid at the back, the clot, the liver, the bile from the gall bladder, and the intestinal contents.

Cultures of B. salmonicida were isolated from all these cultures.

Goldfish number 31.

26/1/32 - mixed 10 cc. of a number 4 McFarland suspension of B. salmonicida with the fish food. Left at room temperature.

1/2/32 - inoculated the fish intraperitoneally with 2/10 cc. number 4 McFarland suspension of B. salmonicida. Left at room temperature.

2/2/32 - found fish floating at 1 P. M. Performed autopsy.

Autopsy of fish.

Exterior examination.

The fish was very bloated in appearance. A small hole was observed at the site of inoculation.

Interior examination.

The intestine was slightly haemorrhagic at the anal end. Otherwise the organs appeared to be in a normal condition. Black spots were found on the liver, and a blood clot on the peritoneum.

Sowed cultures on nutrient agar from the black spots, the liquid at the back, the clot in the peritoneum, the heart, the peritoneal fluid, the intestinal contents, and the bile from the gall bladder.

Cultures of B. salmonicida were isolated from all but the bile.

Other organisms isolated from goldfish.

In nearly every case an organism similar to the Bacillus of the Proteus group referred to by Arkwright was found in the cultures isolated from the fish. These organisms were Gram negative, rod shaped organisms, often coccoid in form, which liquified gelatin rapidly, fermented dextrose, saccharose, maltose, and mannite, but not raffinose, lactose, and inulin. Galactose and laevulose were also fermented. Milk was digested and often became colourless. The organism was also motile, and grew well at room temperature, 28°C. and 37°C.

Table illustrating Goldfish experiments.

Fish No.	Dose	Temperature	Date of Inoculation	Date of Death	Pure cultures from:
1	2/10 cc #1 McF.	Room	30/10/31	3/11/31	abscess, kidney, guts.
2	2/10 cc #1 McF.	Room	30/10/31	Killed 23/11/31	inside of pelvic sore, liver, heart, kidney.
3	2/10 cc #1 McF.	Room	2/12/31	5/12/31	heart, spleen, liver.
4	2/10 cc #1 McF.	Room	2/12/31	11/12/31	heart, spleen, liver, dorsal blood vessel, depth of abscess, clot under the abscess.
5	2/10 cc #1 McF.	Room	2/12/31		
	3/10 cc #1 McF.	26° C.	16/1/32 reinoculated		not affected by the organism.
6	2/10 cc #1 McF.	Room	2/12/31		
	3/10 cc #1 McF.	26° C.	16/1/32 reinoculated	2/2/32	death due to accident no cultures obtained.
7	2/10 cc #1 McF.	Room	2/12/31		
	3/10 cc #1 McF.	26° C.	16/1/32 reinoculated	17/1/32	liver, body cavity, heart.
8	2/10 cc #1 McF.	Room	2/12/31		
	3/10 cc #1 McF.	26° C.	16/1/32 reinoculated	17/1/32	peritoneal fluid, heart, kidney.
9	3/10 cc #1 McF.	26° C.	4/1/32	5/1/32	heart, intestinal contents, haemorrhage near tail, pectoral fin base.
10	3/10 cc	26° C.	4/1/32	5/1/32	inside sore, blood, dorsal blood vessel, red in muscle area, liver, vent.

Fish No.	Dose	Temperature	Date of Inoculation	Date of Death	Pure cultures from:
11	3/10 cc #1 McF.	26°C.	4/1/32	7/1/32	large abscess, muscle opposite inoculation site, intestinal contents, spleen, fluid near back, seared abscess.
12	3/10 cc #1 McF.	26°C.	4/1/32	7/1/32	muscle inoculation point, mid gut, peritoneal fluid, kidney, liver, bile, soft muscle patch.
13	2/10 cc #1 McF.	26°C.	9/1/32		
	3/10 cc #1 McF.	26°C.	16/1/32	17/1/32	peritoneum.
14	2/10 cc #1 McF.	26°C.	9/1/32		
	3/10 cc #1 McF.	26°C.	16/1/32 reinoculated	16/1/32	due to injury to swim bladder-- no cultures obtained.
15	2/10 cc #1 McF.	26°C.	9/1/32		
	3/10 cc	26°C.	16/1/32	17/1/32	peritoneum.
16	3/10 cc #1 McF.	26°C.	16/1/32	17/1/32	kidney.
17	3/10 cc #1 McF.	26°C.	16/1/32	18/1/32	liver, seared abscess, haemorrhage, peritoneal fluid, dorsal blood vessel.
18	3/10 cc #1 McF.	26°C.	21/1/32	22/1/32	black spots on liver, liver, peritoneum, heart.
19	3/10 cc #1 McF.	26°C.	21/1/32	22/1/32	heart, liver, bile, peritoneum, black spots on liver.
20	3/10 cc #1 McF.	Room	21/1/32	22/1/32	kidney, heart, bile, peritoneum, intestinal contents.

Fish No.	Dose	Temperature	Date of Inoculation	Date of Death	Pure cultures from:
21	3/10 cc #1 McF.	Room	21/1/32	22/1/32	heart, peritoneum, kidney, intestinal contents.
22	3/10 cc 1:100,000 dil'n #4 McF.	26°C.	26/1/32	1/2/32	death due to accident-- no cultures obtained.
23	3/10 cc 1:100,000 dil'n #4 McF.	26°C.	26/1/32		
24	3/10 cc 1:100 dil'n #4 McF.	26°C Room	26/1/32 9/2/32	 2/3/32	 posterior intestional contents.
25	3/10 cc 1:100 dil'n #4 McF.	26°C.	26/1/32	3/2/32	death due to accident-- no cultures obtained.
26	3/10 cc 1:100 dil'n #4 McF. then 3/10 cc #4 McF.	Room Room	26/1/32 1/2/32	 2/2/32	 intestinal contents, heart, bluid in back, peritoneum, bile.
27	3/10 cc 1:100 dil'n then 3/10 cc #4 McF.	Room Room	26/1/32 1/2/32	 2/2/32	 heart, peritoneum.
28	3/10 cc #4 McF.	26°C.	26/1/32	28/1/32	fluid in back, haemorrhage in lateral line, peritoneum, blood clot, haemorrhage on right shoulder, haemorrhage on tail, intestinal contents, anus, heart, bile.

Fish No.	Dose	Temperature	Date of Innoculation	Date of Death	Pure cultures from:
29	3/10 cc #4 McF.	26 C.	26/1/32	28/1/32	heart, peritoneum, intestinal contents, haemorrhage.
30	culture fed then 3/10 cc #4 McF.	Room Room	26/1/32 1/2/32	2/2/32	heart, peritoneum, blood clot, liver, bile, intestinal contents, back fluid.
31	culture fed then 3/10 cc #4 McF.	Room Room	26/1/32 1/2/32	2/2/32	peritoneum, black spots on liver, intestinal contents, heart, back fluid.

Experimental Inoculation of Rabbits with B. salmonicida.

Since the organism had remained viable in nutrient broth at 37 °C. for a period of fifty-five days an attempt was made to set up infection in rabbits. Sub cultures were sown from the first broth cultures, again on nutrient broth, and incubated at 37 °C. After two days sub cultures were sown from these on to nutrient broth. These were also incubated at 37 °C. This procedure was continued until five successive sub cultures had been made and incubated at that temperature. From the final sub cultures, cultures were sown on nutrient broth containing a physiological saline solution. These were incubated at 37 °C., and the same procedure followed until five successive sub cultures had been made. Nutrient agar slants were sown from the final tubes and incubated at 37°C. After three days pigment was produced. A number 4 McFarland suspension of the pooled cultures was made in a sterile physiological saline solution.

The following amounts were injected intravenously into rabbits. Injections were made in the ear with a sterile syringe.

Rabbit 1 A - 0.5 cc.
Rabbit 1 B - 0.5 cc.
Rabbit 2 A - 0.5 cc.
Rabbit 5 A - 1.0 cc.
Rabbit 5 B - 1.0 cc.

After one day Rabbit 2 A was found dead and an autopsy performed. The muscle along the posterior surface of the belly was greenish in colour while the pericardial

fluid was bloody. The lungs appeared haemorrhagic, and the blood vessels of the anterior portion of the intestine were engorged. The liver was a dark purple colour. All other organs appeared to be in a normal condition.

Cultures were sown on nutrient agar from the pericardial fluid, the lung, the spleen, the liver, and the heart. These were obtained in the following manner. The organ was seared, then cut with a sterile knife. A sterile Pasteur pipette was forced into the cut, the fluid drawn into the pipette, and sown on the agar slants. At the same time smears were made from all these organs and stained with Gram's stain. On examination these were found to contain tissue cells only. The cultures were incubated at 37°C. All proved to be sterile with the exception of that from the pericardial fluid. The only organism isolated from it was a Gram negative bacillus which appeared to form spores, and which showed a white wrinkled growth on nutrient agar. Its action on carbohydrates was not at all similar to that of B. salmonicida.

dextrose	+	in 1 day.
maltose	+	in 1 day.
sucrose	+	in 1 day.
salicin	+	with pellicle formation in 2 days.
xylose		slight acidity with pellicle formation in 2 days.
mannite	+	with pellicle formation in 2 days.
raffinose	+	with pellicle formation in 2 days.

The other rabbits were not affected in any way by the intravenous inoculation with B. salmonicida. As Rabbit

2 A had previously been in ill health death at this time was probably the result of shock.

Influence of the organic content of water on the organism, B. salmonicida.

Preparation of water of varying organic content.

On 9/2/32 obtained samples of sewage from the City Sewers Department. Until used this was kept in corked flasks in the ice box. After 12 hours standing the effluent was decanted off and the solid material disposed of. Three 500 cc. samples of effluent were kept for chemical analysis and eighteen 1,000 cc. flasks were prepared in the following manner.

Sets, numbers 1 and 3	Set number 2
Flask C - 500 cc. sterile tap water.	Flask C - 500 cc. sterile tap water.
Flask 0 - 500 cc. undiluted effluent.	Flask 0 - 500 cc. undiluted.
Flask 1 - 500 cc. 1:10 dilution effluent in sterile tap water.	Flask 1 - 500 cc. 1:10 dilution effluent in sterile tap water.
Flask 2 - 500 cc. 1:100 dilution effluent in sterile tap water.	Flask 2 - 500 cc. 1:100 dilution effluent in sterile tap water.
Flask 3 - 500 cc. 1:1000 dilution effluent in sterile tap water.	Flask 3 - 500 cc. 1:1000 dilution effluent in sterile tap water.
Flask 4 - 500 cc. 1:10,000 dilution effluent in sterile tap water.	Flask 4 - 500 cc. 1:10,000 dilution effluent in sterile tap water.
Flask 5 - 500 cc. 1:100,000 dilution effluent in sterile tap water.	Flask 5 - 500 cc. 1:100,000 dilution effluent in sterile tap water.
Flask 6 - 500 cc. 1:1,000,000 dilution effluent in sterile tap water.	Flask 6 - 500 cc. 1:1,000,000 dilution effluent in sterile tap water.

Sets, number 1 and 3 were then sterilised, while set number 2 remained non-sterile.

Chemical analysis.

Placed the three 500 cc. sewage effluent samples in large beakers and evaporated over a water bath, washing down all deposits using tap water and a rubber policeman, until all had become a thick, syrupy mass. Poured these samples into carefully weighed and dried crucibles, put on water bath, and evaporated until almost dry. Then dried in an oven at 103°C. for half an hour, weighing and repeating until a constant weight had been reached. Finally heated crucibles slowly at first over a clay triangle with a bunser burner, raising the temperature gradually until a red heat had been reached. Kept at this temperature for 30 minutes, cooled and weighed. Repeated the procedure, until a constant weight was reached. The samples were kept in a desiccator, when not in use.

Sample A.

Constant weight of empty crucible.....31.1320 grams.

Weight of crucible + solids.....31.7872 grams.

Weight of solids..... .6552 grams.

Constant weight of crucible + ash.....31.3555 grams.

Weight of ash..... .2235 grams.

Weight of solids - weight of ash..... .4317 grams.

Sample B.

Constant weight of empty crucible.....31.8420 grams.
Weight of crucible + solids.....32.495 grams.
Weight of solids..... .6530 grams.
Constant weight of crucible + ash.....32.0570 grams.
Weight of ash..... .2150 grams.
Weight of solids - weight of ash..... .4380 grams.

Sample C.

Constant weight of crucible.....33.0044 grams.
Weight of crucible + solids.....33.6571 grams.
Weight of solids..... .6527 grams.
Constant weight of crucible + ash.....33.2450 grams.
Weight of ash..... .2414 grams.
Weight of solids - weight of ash..... .4113 grams.

Results of analysis.

Sample A - The weight of organic solids in 500 cc. undiluted effluent was .4317 grams or 431.7 mgm. which would be 863.4 mgm. per litre.

Sample B - The weight of organic solids in 500 cc. undiluted effluent was .4380 grams or 876 mgm. per litre.

Sample C - The weight of organic solids in 500 cc. undiluted effluent was .4113 grams or 822.6 mgm. per litre.

The average weight of organic solids in 1 litre undiluted effluent was then 854.0 mgm.

Then in a 1:10 dilution of effluent there would be 85.4 mgm. organic solids per litre of solution.

In a 1:100 dilution of effluent there would be 8.54 mgm. organic solids per litre of solution.

In a 1:1,000 dilution of effluent there would be .854 mgm. organic solids per litre of solution.

In a 1:10,000 dilution of effluent there would be .0854 mgm. organic solids per litre of solution.

In a 1:100,000 dilution of effluent there would be .00854 mgm. organic solids per litre of solution.

In a 1:1,000,000 dilution of effluent there would be .000854 mgm. organic solids per litre of solution.

Testing the Inhibitive Action of Organic Material Upon the Growth of B. salmonicida.

Method.

To each of the 18 litre flasks were added 0.2 cc. of a number 1 McFarland suspension of pooled three day old pigmenting cultures of B. salmonicida isolated from goldfish. This amount had previously been found to be the amount which would form from 300 to 400 colonies on nutrient agar plates after one day when sown in 500 cc. sterile tap water. All flasks were left at room temperature. Plates were sown daily from each flask, using a 1 cc. sample, after first thoroughly shaking each flask.

Results.

(a) Set number 2.

This work may be inaccurate because of the difficulties experienced in ascertaining which were the typical B. salmonicida colonies. Many small, circular, translucent colonies were observed which were not of that type.

The plates were made daily for thirty-five days, and each day were crowded with growth. The growth of the B. salmonicida colonies seemed to increase daily and far exceeded the original limit of 300 to 400 colonies. After fifteen days the numbers of B. salmonicida colonies showed a decline on all plates, and continued to decline daily. It appeared as if the growth of B. salmonicida was more

prolific on the higher dilutions, while more growth of the sewage organisms appeared on the lower dilutions.

Growth on the control plates was absent after eleven days.

(b) Sets numbers 1 and 3.

The appearance and extent of the growth on the plates made from these sets of flasks can be explained most readily by the table and the graph on the following pages.

Growth on the plates sown from the control flasks of sterile water was absent after eleven days, after showing a gradual decline.

On the plates sown from the flask of undiluted effluent growth was prolific from the first day until the end of thirty-five days. A daily increase was noted in the growth from these flasks.

In each of the diluted solutions of effluent the number of colonies gradually increased, suddenly reaching a high uncountable limit. This limit was reached in ten days in the 1:10, the 1:100, and the 1:1,000 dilutions, while it was not until after seventeen days that this peak was reached in the case of the 1:10,000, the 1:100,000, and the 1:1,000,000 dilutions. After thirty-one days the number of colonies on all plates still remained uncountable, though a gradual decrease in the number of colonies was noted on plates poured from dilutions 1:10,000, 1:100,000, and 1:1,000,000 after twenty-three days. A decrease in the number of colonies

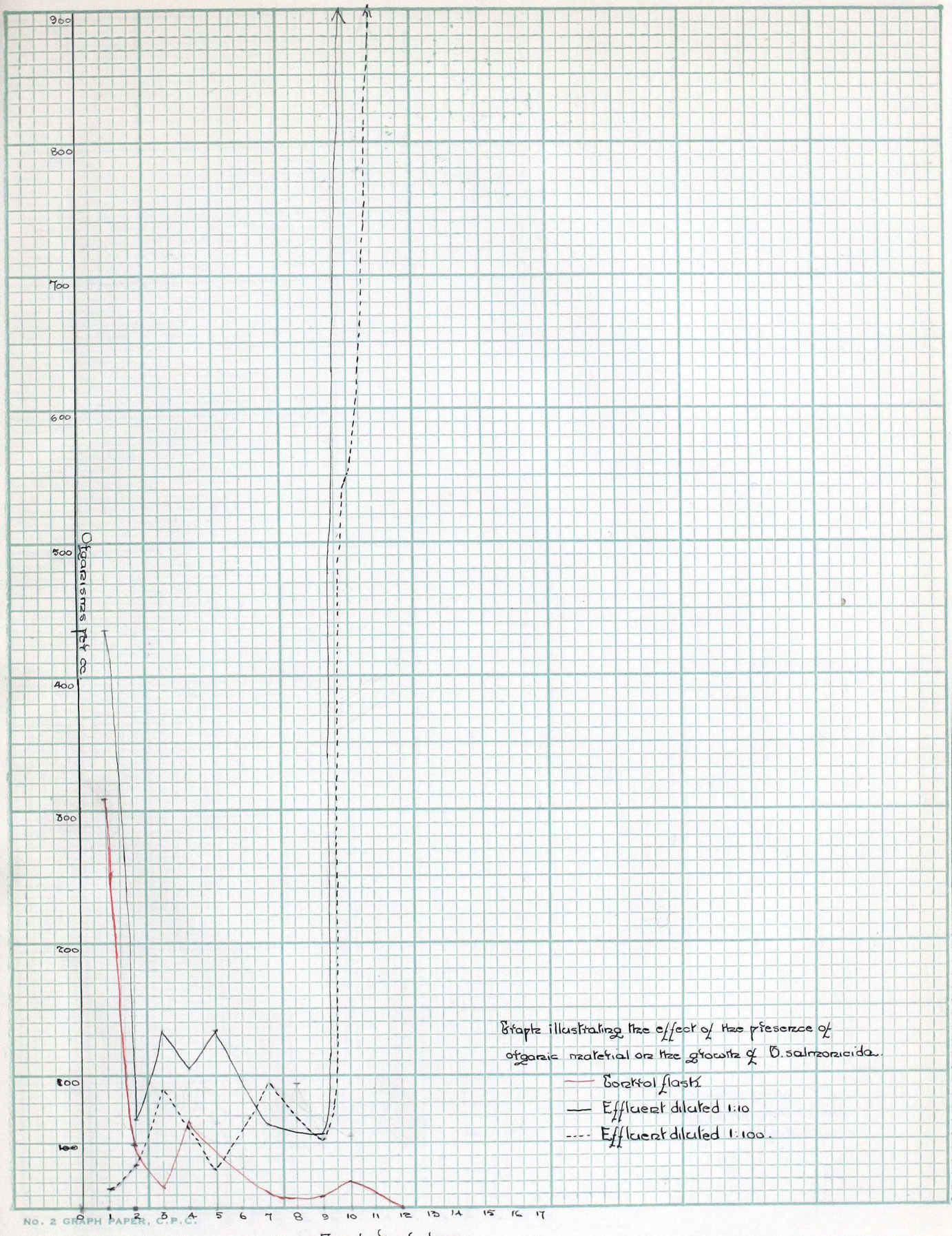
on plates poured from dilutions 1:100 and 1:1,000 was observed after twenty-nine days.

Unfortunately because of time shortage this work was not completed.

Record of growth on plates of set number 3.

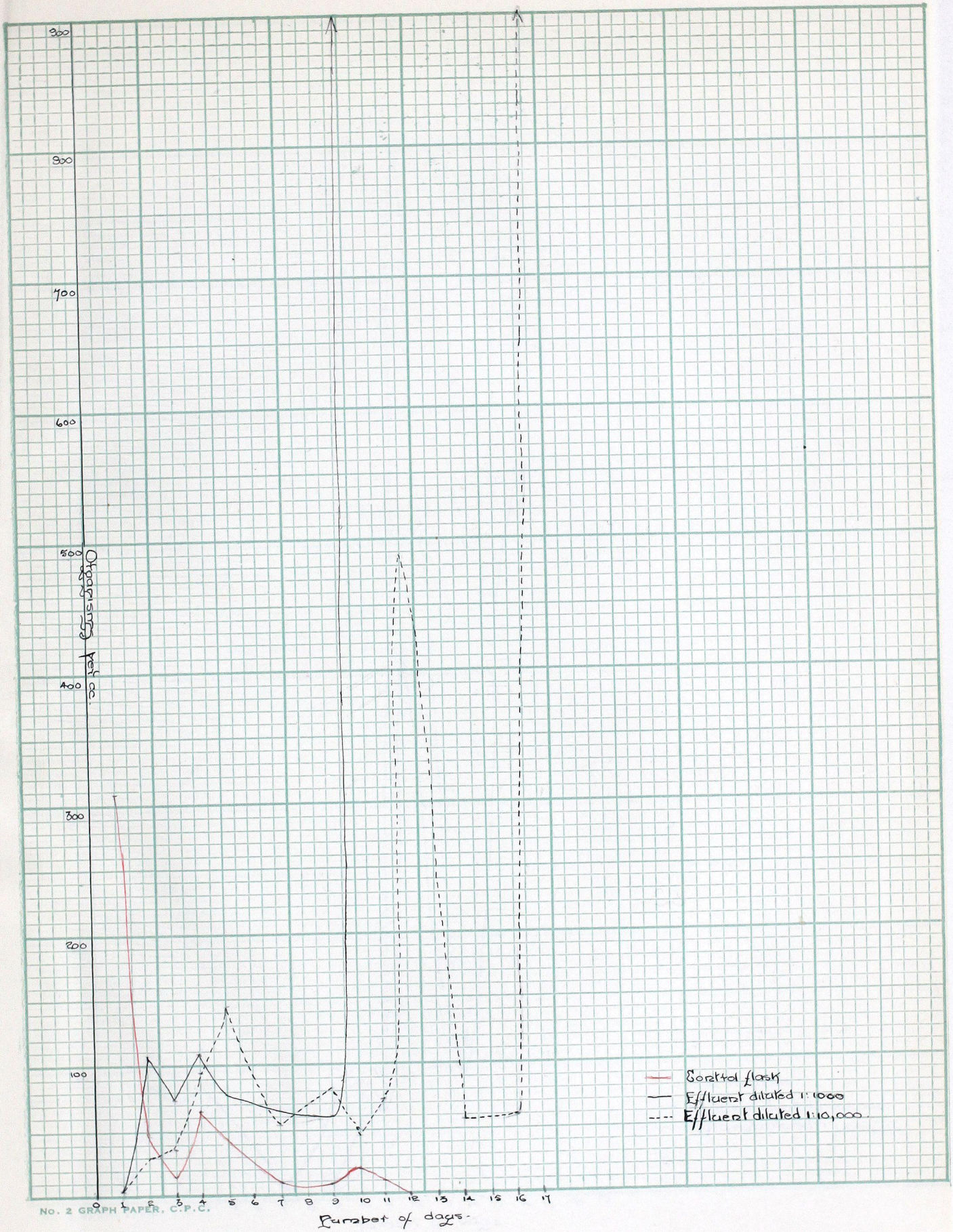
(recorded as organisms per cc.)

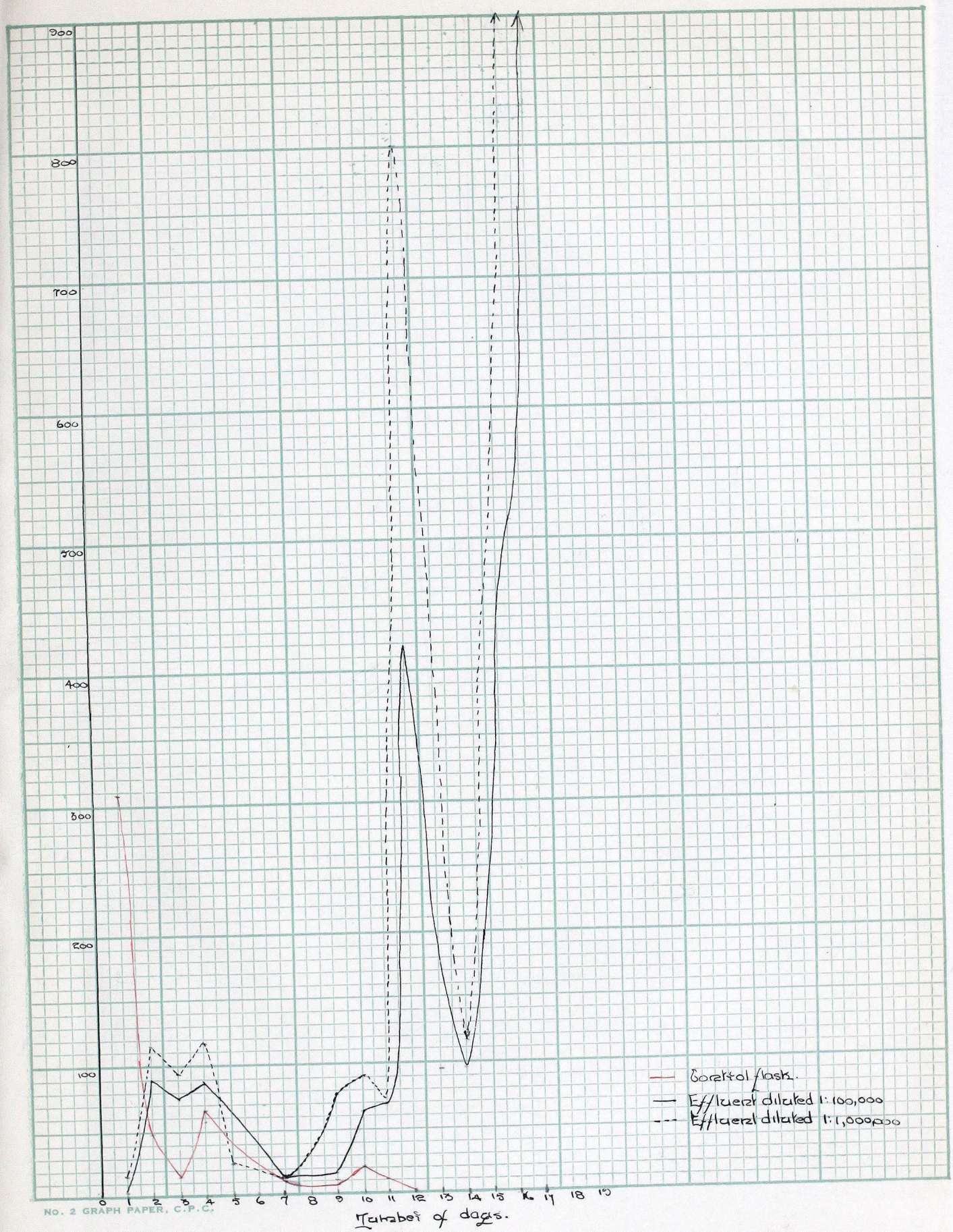
Number of Days	Flask C Sterile Water	Flask 0 854 mgm. organic solids per L.	Flask 1 85.4 mgm. organic solids per L.	Flask 2 8.54 mgm. organic solids per L.	Flask 3 .854 mgm. organic solids per L.	Flask 4 .0854 mgm. organic solids per L.	Flask 5 .00854 mgm. organic solids per L.	Flask 6 .000854 mgm. organic solids per L.
1	309	uncountable	435	13	2	2	2	14
2	49	"	69	54	107	30	90	113
3	15	"	134	90	73	46	74	94
4	64	"	105	63	108	95	86	114
5	no record of plate	"	133	30	77	144	no record of plate	25
7	10	"	62	95	64	54	13	11
9	7	"	57	53	60	81	14	78
10	20	"	uncountable	550	over 1000	45	62	90
11	10	"	"	uncountable	uncountable	72	68	71
12	0	"	"	"	"	487	418	800
14	0	"	"	"	"	57	95	115
16	0	"	"	"	"	60	512	over 1000
17 - 30	0	"	"	"	"	uncountable	uncountable decrease after 23 days	uncountable decrease after 23 days
				decrease after 29 days	decrease after 29 days	decrease after 23 days		



Graph illustrating the effect of the presence of organic material on the growth of B. salmonicida.

Control flask
Effluent diluted 1:10
Effluent diluted 1:100.





Conclusion.

1. Carbohydrate reactions.

On glucose the B. truttae of Marsh produced gas without sugar fermentation. Arkwright, working on B. salmonicida, found both acid and gas production in that sugar. Neither Plehn nor Davis described the carbohydrate reactions of B. salmonicida. Williamson reported the production of acid with gas in some instances, in glucose and mannite. She also stated that saccharose and lactose were not fermented but that the medium became alkaline.

The strains isolated from the Elk river fish and the goldfish produced acid, with occasionally a slight amount of gas production, in both mannite and glucose tubes. In saccharose, lactose, raffinose and inulin the medium became alkaline and the growth was abundant. In laevulose, salicin, galactose, maltose, arabinose and xylose the sugar was fermented with acid production in all cases.

2. Pigment solubility.

Marsh reported that the pigment of his B. truttae was soluble in alcohol, while Arkwright found that "if absolute alcohol were poured on to a moist pigmented agar culture the watery solution of the pigment diffused out into the alcohol, and that the pigment remained in solution in the impure alcohol."

The strains of B. salmonicida used in these experiments produced a pigment which diffused from the medium into

the alcohol, and remained in solution in the alcohol.

3. Reaction of medium - its effect on the growth and pigment production of B. salmonicida.

Marsh's B. truttae was very sensitive with regard to the reaction of the medium on which it was cultivated while Williamson found the optimum pH. for pigment production to be pH. 7.6.

Pooled strains of B. salmonicida grew on agar slants at room temperature within a pH. range of 5.0 to 9.2 inclusive. Pigment was produced within a pH. range of 5.2 to 9.2 inclusive. After seven days growth without pigment production on an agar slant of pH. 5.0 when sub cultures were sown on agar with a pH. of 7.0 pigment was again produced in three or four days.

4. Viability of the organism.

Workers seem to agree that clear water is deleterious to the development and growth of the furunculosis organism. Plehn found that pure water was disadvantageous to the bacterium, that in it B. salmonicida was dead after three days, while Arkwright found it alive, though in diminished numbers, after five days in sterile water. Dr. Arkwright also observed that sea water was injurious to the growth of the organism, that the organism cannot be recovered after nineteen hours.

Our strains of B. salmonicida did not live long in sterile distilled water. In sub cultures on nutrient agar

plates, in some instances no growth was reported after one day. In other cases the organism remained alive for three days, and in one instance for four days.

In sterile tap water the organism survived for a longer period. Even after eleven and twelve days colonies were found on nutrient agar plates. These were in very small numbers. In other flasks containing the same amount of sterile tap water the organism could not be recovered after two or three days.

Arkwright found that in distilled water containing 3% sodium chloride B. salmonicida soon died. Using the strains of B. salmonicida isolated from goldfish the organism was recovered in small numbers after eight days in 3% salt water. No growth was reported on the ninth day. In 1% salt water the organism remained alive for nine and eleven days, while in .85% salt water the average time reported was ten days.

It would seem then, that neither clear water nor water containing sodium chloride are particularly favourable for the growth of B. salmonicida. The short time that it can remain alive in such water however may be time enough for the organism to be carried to many fish, thus causing infection.

5. Temperature.

Arkwright reported that the organism soon died when incubated at 36°C. Davis secured similar results, stating that the organism cannot live long at 37°C. Williamson found no growth at 37°C.

Pooled strains of B. salmonicida isolated from goldfish could survive over a period of from sixteen to eighty days at 37°C. When sub cultures were made from these, rapid growth took place at the same temperature. Pigment was also produced by these cultures at 37°C.

The organism remained viable at 28°C. and at 14°C. for at least three months. Pigment production also took place at these temperatures.

It may be that the organism in its passage through the more resistant goldfish, and its incubation at 26°C. had become fortified to a greater extent against changes in temperature.

6. Relation to oxygen.

The organism, according to Williamson, is an aerobe and a facultative anaerobe. No conclusions can be drawn from our work.

7. Pathogenicity Experiments on Fish.

From the inoculation experiments it would appear that B. salmonicida when injected intraperitoneally or intramuscularly into goldfish is able to produce septicaemia and death, in some cases with the presence of local abscesses and haemorrhages, in others with no such symptoms.

Feeding and scarifying experiments did not seem to affect goldfish in any way, perhaps because of the hardy nature of the fish. Certain of the fish were able to recover after what appeared to be a typical attack of furnculosis--take for example goldfish number 2 which after twenty-five days showed signs of recovery, while goldfish numbers 5 and 6, in spite of two inoculations with the organism, and a change from room temperature to 26°C., were both able to resist the disease. Goldfish number 5 appeared active and in good health throughout the year.

Suspensions of the organisms in very high dilutions did not seem to affect the fish, witness goldfish number 23 which remained active despite its inoculation with a 1:100,000 dilution of a number 4 McFarland suspension of the organism.

In the cases of goldfish numbers 7 and 8 which were resistant to a first inoculation and incubation at room temperature, reinoculation and incubation at 26°C. seemed to decrease this power of resistance. Goldfish numbers 13 and 15 were unaffected by a first inoculation with incubation at 26°C. but quickly succumbed after a second dose.

Inoculation with incubation at 26°C. seems to cause more rapid death with exterior symptoms of the disease at the same time, while at room temperature death was often caused just as rapidly but without the appearance of these

external signs. As an exception to the rule, however, we have goldfish number 20 and 21 at room temperature, and numbers 18 and 19 at 26°C.

Laboratory experiments show, then, that the pigment forming bacterium B. salmonicida is able to produce many of the most characteristic features of furunculosis as described by Mettam, Arkwright and Plehn, namely abscesses, haemorrhagic areas in the muscles, congestion of the intestine, particularly the lower, and a discharge of a purulent liquid from the anus. There are cases when some or all these symptoms were not observed but where death occurred and the injected organism was recovered from the fish.

8. Pathogenicity Experiments on Rabbits.

Though B. salmonicida had been grown on both nutrient broth, nutrient agar and on nutrient broth containing a physiological saline solution for some time at 37°C. when injected into the marginal ear vein of rabbits it had no apparent effect on the rabbits during a period of twenty-two days.

The habitat of the organism, then, seemed to be restricted to the cold blooded animals where it is an active parasite circulating with the blood and attacking the tissues of the host. Even here its activity is restricted for, according to most workers, B. salmonicida is not pathogenic to frogs. No work was done on amphibians in connection with this research.

9. Effect or organic material on the growth of the organism.

Plehn said that though the furunculosis bacterium died in clear water within one to three days B. salmonicida would grow in the presence of a great deal of contamination in the water. Furunculosis as it occurs in sewage polluted waters and in water containing disposals from breweries and distilleries is commonly more infections and more disgusting than that found in clear water, according to Plehn. The results of her work show that the numbers of B. salmonicida increased by millions in water rich in organic matter, in the same time required for it to die out in the same amount of clear water.

Williamson, in 1928, found no growth of B. salmonicida in sewage after forty-eight hours, but that warm weather and low water favour the development of B. salmonicida.

Horne found no growth of the organism in sewage after forty-eight hours, while Davis remarked that the organism "may live for weeks in water containing an appreciable amount of organic matter".

From our experiments it would appear that, when sown in non sterile sewage polluted water, the organism first showed a rapid increase in numbers, but that after ten days there was a gradual decrease in the numbers of B. salmonicida. On the other hand, in sterile sewage polluted water there was first a decline in the numbers of the organism present, then in ten days there was a rapid

and unaccountable increase in numbers in the lower dilutions. In the undiluted effluent there was no lag phase but the numbers increased daily. In the higher dilutions the increase in numbers did not take place until the sixteenth or the seventeenth day in the 500 cc. samples. The numbers of the organisms still remained at an uncountable height even after thirty days, but after twenty-one days a slight decrease in the numbers was noticed, and the growth of the organisms became slower.

Unfortunately there was not time enough left to carry these experiments further, though many new and interesting problems presented themselves for future study.

Summary of the characteristics of the Elk River strain of *B. salmonicida*.

1. *B. salmonicida* is a pleomorphic Gram negative organism staining easily with ordinary aniline dyes.
2. *B. salmonicida* grows well on ordinary nutrient agar, nutrient broth, Loeffler's blood serum, gelatin and litmus milk. It produces haemolysis on blood agar plates, does not produce indol, and is a moderate starch lover.
3. The pigment produced by *B. salmonicida* is soluble in water and diffuses out into an alcohol solution.
4. *B. salmonicida* does not thrive on a medium of distilled water, sterile tap water, or sterile tap water containing sodium chloride.
5. *B. salmonicida* strains isolated from goldfish will grow for some time when incubated at 37°C., 28°C., and 14°C.
6. *B. salmonicida* produces no pigment in the absence of oxygen.
7. *B. salmonicida* is pathogenic for goldfish, producing lesions and death in most cases, but not for rabbits.
8. *B. salmonicida* grows abundantly in sewage polluted water and remains viable for at least thirty-one days.

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