BACTERIAL INFECTIONS

OF

CERTAIN LOWER VERTEBRATES

with particular reference to

"FURUNCULOSIS OF THE SALMONIDAE"

A Thesis

Thesis submitted for Degree of Ph.D.

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Bacteriology Department,
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by

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TABLE OF CONTENTS.

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I. INTRODUCTION AND REVIEW OF LITERATURE.

Comparatively little is known regarding the bacterial diseases of the lower vertebrates, in con-:trast with our knowledge of such conditions in mammalian animals. While disease among the cold-blooded vertebrates may be of relatively little econ-:omic importance, the study of microbic infections in these animals is of great interest, both in general biology and also from the comparative point of view.

Much can be learned by the study of biological processes in the lower forms of life, and there is still considerable scope for the study of bacterial infections in the lower animals.

Bacterial diseases of fish have received attent-:ion from time to time, and these are often of con-:siderable economic importance. The largest section of this thesis deals with "Furunculosis of the Salmon-:idae", a disease which has been known since 1894, and has attracted much attention. The writer has studied the biology of the causative organism, and the associated pathological condition has been found to be a good example of general infection, which seems to be typical of bacterial disease in cold-blooded animals./

animals.

When the literature on diseases of fish is consulted, it is found impossible to identify the bacteria described in the older records, but the general nature of the infections described is easily recognised.

In 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 yellowish discharge from the cloaca. The condition was that of a general infection, in which bacteria appeared in the blood during life. Small superficial haemorrhages were seen, and there were degenerative changes in the muscle, skin, and fins. From the description, a secondary infection, possibly the same as that now recognised as due to Saprolegnia ferax, (Hume Patterson, 1903) appeared to have taken place, as patches of white mossy substance were seen on the skin. Haemolysis apparently took place in the blood vessels, and the investigators described bacteria as being present in the blood during life, but disappearing rapidly after death. The organisms seen were short spindle-shaped rods, often in pairs, and slightly motile, and smaller spherical or kidney-shaped structures with a vibratile movement, which were thought to/

to be vibrios. The mode of infection was not dis-:covered. Healthy fish were kept in tanks beside diseased fish and did not become infected. Frogs and warm-blooded animals were not infected by contact with contaminated water, nor by being fed with diseas-:ed fish.

Fabre-Domergue (1890) described a tumour-like lesion in Caranx trachurus, and stated that the les-:ion was full of bacteria. The organisms were often seen in pairs, and the larger forms were twisted spirally. He made cultures on fish-gelatin, which was liquefied and assumed a green fluorescence. The bacteria were, he thought, slightly Gram-positive. Experimental inoculation of cultures into several fish was unsuccessful. It is certain that Fabre-Domergue did not obtain a pure culture, but B. fluor-:escens seems to have been present, and the spiral organism may have been a water vibrio or spirillum. Probably the lesion was a connective tissue tumour, which is not uncommon in fish (Johnstone, 1915), and which had undergone degeneration and been invaded by water organisms.

From 1892 for a few years great attention was given to epidemics of bacterial disease 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 substance toxic for both cold- and warm-/

warm-blooded animals. Charrin (1893) isolated several bacilli, one of which proved pathogenic, from diseased fish in the Rhône. This organism was a motile bacillus, which grew at 20°C. but not at 37°C. It liquefied gelatin, produced acid and clot in milk, and bouillon culture smelled of trimethylamine. Fish became infected from water contaminated with the bacillus, and haemorrhages occurred in the muscles and skin. Toxins were produced and these were pre-:cipitable by alcohol. The toxins were virulent for warm-blooded animals as well as for fish. Charrin referred to the fact that he had noticed that infect-:ions in fish usually caused intra-muscular haemorr-:hages, and he had produced such symptoms in fish by inoculation with B. pyocyaneus.

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 intra-:muscular lesions, general congestion, and blood in-:fection. The organisms isolated from trout, frogs, and crayfish all had the same characters and were all pathogenic for these animals. Infection occurred from the water without previous lesions. A toxin precipitable by alcohol was formed. This organism,

from the damage it did among fresh water animals, was known as "La Peste des Eaux douces". Bataillon thought that it belonged to the group "Termo" (Proteus). Probably the bacillus isolated by Charrin was the same as this.

Mercier and Lasseur (1911) also dealt with "La Peste", and described the causative organism as Gram-negative, pleomorphic, and motile. Fish, frogs, and crayfish were susceptible, and a filterable toxin was produced. Lasseur stated that the bacillus was B. chlororaphis (Guignard and Sauvageau, 1894), and pro-:duced crystals of chlororaphine.

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

Wyss (1898) studied a disease of Leuciscus rutilus, in which haemorrhagic lesions were formed, and a bacillus was isolated from the blood and all organs. This organism was rod-shaped or diplo-coccal, Gram-negative, liquefied gelatin and formed a pellicle on bouillon. It was pathogenic for fish, mice, and guinea pigs. Wyss identified the organism as B. vulgare (Proteus).

A bacillus which caused ulcerative septicaemia in gold fish was described by Ceresole (1900). He said/

said that it was faintly Gram-positive, and showed vestiges of spores. This organism was virulent for rabbits as well as for fish.

An epizootic disease affecting many fish was investigated by Babes and Riegler (1903) at Bukarest. A pathogenic organism was isolated from the fish and also from the water, and had the characters of the Proteus group. Its optimum temperature was 20°C. Gelatin was liqufied, there was a brownish growth on potato, the medium became greenish, and a pellicle was formed on bouillon which had the smell of trimethylamine. The diseased fish showed superficial ecchymosis, and the muscle was necrotic in parts. The bacillus was named Proteus piscicidus versicolor.

About the same time as these diseases of fish were being investigated, very similar conditions in frogs were receiving attention. Ernst (1890) isolated a bacillus from frogs during an epizootic among these animals at Heidelberg, and called it B. ranicida. It was Gram-negative, non-sporing and slender. The optimum temperature for growth was 20°C. - 30°C., and frogs were more susceptible at low temperatures. Gelatin was liquefied, and a greenish colour appeared on that medium, on agar, and on potato, but this was not fluorescent. Rana esculenta was more susceptible than Rana temporaria. Inoculation of cultures into the dorsal lymph sac of frogs produced death by blood infection in two to five days.

Sanarelli/

Sanarelli (1891) studied a similar disease in frogs, but the organism he isolated produced a yellow-:ish pigment, and was pathogenic for rabbits. This organism he called B.hydrophilus fuscus.

Roger (1893) isolated an organism which he identified as B. hydrophilus fuscus, from frogs in Paris. It produced a general infection in cold-blooded animals and was also virulent for rabbits. In these, muscular and intestinal haemorrhages occurr-:ed, and in guinea pigs there was haemorrhagic oedema, unless death from septicaemia was very rapid. Gen-:eral blood infection always took place.

In the same year Trambusti (1893) also found B. hydrophilus fuscus in frogs, and obtained from that organism a toxin, precipitable by alcohol and soluble in water, which produced paralysis, and death if administered in sufficiently large quantities, in experimental animals.

Russell (1898) and Emerson and Norris (1903) in America studied a disease of frogs known as "Red Leg", and this appears to be the same as that recorded by Sanarelli. However, Emerson and Norris found that their organism differed from that of Sanarelli by not producing yellow pigment, and not being pathogenic for rabbits. Russell's strain was identical with B. hydrophilus fuscus, and rabbits were susceptible to it. Russell states that the bacillus was motile by a single polar flagellum.

Vénulet and Padlewski (1913) described an organ-:ism pathogenic for frogs, not differing in essentials from B. hydrophilus fuscus, and which they called B. septicæmiae ranarum. It had a single polar flag-:ellum, and was pathogenic for both cold- and warm-blooded animals, in which a general infection was produced. Filterable toxins were found to be viru-:lent for guinea pigs and mice.

In all these cases of disease in frogs, the causative organisms were also isolated from the water supply. A great resemblance will be seen between these organisms, both those isolated from diseased fish and those from frogs. Some of the investigat-:ors thought that the bacilli they described formed spores, and there was doubt in cases as to whether the organisms were Gram-positive or negative, but these differences were probably due to the staining technique. The main differences were in pathogenic-:ity for rabbits (which was not tested in all cases), and in production of greenish or yellowish pigment. It appears quite probable that the organisms involved were very closely related, if not strains of one species. But this cannot be settled for want of data.

Bergey, in his Manual of Determinative Bacteriol-:ogy, places B. hydrophilus fuscus in the Proteus group, which appears to be a mistake, for although Sanarelli did not mention the position of flagella in/

in the bacillus, Russell, who isolated a bacillus identical with Sanarelli's, described one polar flagellum. Bergey calls the organism Proteus hydrophilus fuscus, and describes it as having peritrichous flagella.

Several cases of ulcerative disease in sea fish have been described. Johnstone (1905) recorded an outbreak of disease of plaice at Port Erin, in which the lesions were spreading ulcers. He came to the conclusion that the condition was due to infection by fungi, as in that year and in 1916 he found fungal colonies in the viscera, especially the liver, of affected fish.

Riddell and Moore Alexander (1912) also investigated this disease of plaice at Port Erin. There was great destruction of tissue, and the ulcers extended into the muscle in many cases. Three organisms were isolated:

(1) Isolated from ulcers, liver, and heart blood; a thick and curved Gram-negative bacillus, sometimes occurring in long straight forms; optimum temperature was 20°C.; the organism died out in a few days at 37°C.; agar colonies were yellowish, gelatin was liquefied, and milk and sugars were unchanged.

(2) Isolated from ulcers of some fish and from the water from which they were obtained; this organism was thin, curved, and Gram-negative. The optimum/

optimum temperature was 20°C., and viability was rapidly lost on culture media.

(3) Isolated from heart blood of one fish and from water; this organism was like B. salmonis pestis (Hume Patterson, 1903), but was not viable in salt water.

An ulcerative disease affecting haddocks, whiting, and plaice at the Bay of Nigg hatchery was described by Anderson (1909). Some of the haddocks and whit-:ing were caught near a sewer and put in tanks for study. These fish had small ulcers on the skin, and in captivity the lesions rapidly spread, while other fish in the tanks also became infected. Water from these tanks reached others containing plaice, which then contracted the disease. The condition was rapidly fatal. A coccus corresponding in characters to Staphylococcus pyogenes aureus, and typical B. coli were obtained in culture from the ulcers. In a few cases the heart blood gave pure cultures of the coccus. In addition to these two organisms, actively motile, vibrio-like bodies were seen in fresh hanging-drop preparations made from the ulcers. This organism, however, did not appear in fixed and stained prepar-:ations, nor did it grow on culture media. Anderson considered it probable that the fish had been infect-:ed from the sewage.

Drew (1909) described bacterial infection of the swim-bladder of trout subsequent to invasion of that organ/

organ by a thread-worm. There were many kinds of bacteria involved, but they were not described. The walls of the bladder showed fibrinous exudate and leucocytosis. In the same paper Drew recorded a case of pericarditis in a turbot, and described an organism isolated from the pericardial fluid. This was a slender, Gram-negative diplo-bacillus, which produced cream-coloured colonies; gelatin was not liquefied.

Drew also mentioned "Salmon Disease", which affected trout, chubb, roach, dace, and eels in the Colne. He isolated Saprolegnia ferax and B. salmonis pestis (Hume Patterson, 1903) from these cases.

There are two diseases of the Salmonidae which are of great importance. One is the so-called "Salmon Disease", which takes the form of ulceration, especially on the head, and degeneration of the fins and tail. A fungus, Saprolegnia ferax, is found growing on the diseased fish, and was for long con-:sidered to be the cause of the condition. Hume Patterson (1903) investigated this disease and isol-:ated a gelatin-liquefying bacillus which was Gram-negative, non-sporing, and produced a cream-coloured growth on agar. This organism was pathogenic for fish, but not for frogs, mice, or guinea pigs. It did not infect fish unless there was a skin lesion, but this might be extremely small. Necrosis of the muscle tissue at the site of infection occurred, and the/

the bacillus was not found in heart blood, but only in the vicinity of lesions. Hume Patterson called the organism B. salmonis pestis. It was found in all cases of the disease investigated, and a similar pathogenic condition was produced experimentally in fish. Saprolegnia ferax is commonly found in water, readily grows on any dead organic matter, and appar-:ently settles on necrotic areas of the skin, after the bacillus has formed these lesions. Hume Patter-:son did not explain how death was caused, and unless toxin is produced by the bacillus, this is difficult to explain, since as described by him, infection is local, not general. All fresh-water fish appear to be susceptible to this disease, but the Salmonidæ more than others.

An epizootic disease affecting the Salmonidae, and characterised by boil-like superficial lesions, was first described by Emmerich and Weibel in Germany in 1894, and called "Furunculosis" from the appear-:ance of the lesions. Plehn (1909) recorded a dis-:ease which she identified as Furunculosis occurring in Southern Germany in 1909 and subsequent years, and she stated that previous to this date the disease chiefly affected fish in fish-farms, only becoming prevalent afterwards in natural waters.

In America a disease of trout was studied by Marsh in 1902, which is now believed to have been Furunculosis/.

Furunculosis. Surbeck described an outbreak of Furunculosis in Switzerland in 1909, and Drouin de Bouville, in France in 1910, also reported the occurr-:ence of the disease.

The first occurrence of this disease in Britain was recorded in England by Masterman and Arkwright in 1911, while Mettam described an outbreak in Ireland in 1914. The disease appears to have been present in Scotland for some years, but there was no official record before 1926.

Furunculosis is a disease of the Salmonidae, but there have been suggestions, by Plehn (1909) and others, that other fresh water fish may be attacked, especially when an epizootic is at its height, and the virulence of the specific causative organism is increased. Outbreaks appear to be isolated, but once the disease has appeared in a district it tends to persist. In general it has been noted that warm weather and low water are favourable to the condition.

There are suggestions that susceptibility varies among different species of the Salmonidæ, and also among individuals of different age in the same spec-:ies. Salmo salar and Salmo truttæ are very suscept-:ible, while Salmo irideus is relatively immune. The physical condition of Salmonidae varies at different ages, which may explain certain of the variations in susceptibility referred to.

Emmerich and Weibel (1894) isolated from furun-:culous/

furunculosis fish a specific bacillus, which they called Bacillus salmonicida, and described as a short rod-shaped organism, non-motile, non-sporing and Gram-negative. It grew well on ordinary media at room temperature, producing a diffusible brown pigment in old cultures on nutrient agar, and in gelatin stab culture liquefied the medium in a manner believed to be characteristic of the organism, as liquefaction was stated to proceed from below upwards, with pro-:duction of gas funnels. These two characters were regarded as criteria for identification of B. salmon-:icida, and Hofer (1904) and Drouin de Bouville (1910) also considered them to be of primary importance. However, other workers - Plehn (1909), Marsh (1902) and Arkwright (1911) - have observed that the mode of liquefaction of gelatin is not constant for all strains of the bacillus, and the appearance of the funnel of liquefaction does not differ from that formed by some other bacteria. Production of diffusible brown pig-:ment is not common as a bacterial characteristic, but it is not peculiar to B. salmonicida.

Although the cultural tests used by earlier work-:ers are not sufficiently exact and critical, there is little doubt that the bacillus described by all those who have investigated furunculosis in Salmonidæ is the same as the B. salmonicida of Emmerich and Weibel.

The pathology of "Furunculosis" has been little understood,/

understood, and the name is misleading. There is no leucocytic infiltration such as we find in "boils" in warm-blooded animals, and the lesions are not "furun-:cles", but areas of necrosis. Arkwright recognised this, but other workers believed that the lesions con-:tained pus. Murisier (1910) found great leucocytic infiltration, and compared what he saw in this dis-:ease of fish to the inflammatory reactions occurring in certain brain lesions in the human subject; but as he did not describe the naked-eye appearances, nor the causative organism, it seems doubtful whether the condition he investigated was **Furunculosis.**

The reddish, pus-like contents of the lesions, which are described in most cases, consist of necrotic muscle fibres, free blood cells and bacteria.

The disease is essentially a general blood infect-:ion. Plehn states that there are three forms: first, one in which there is an "incubation period"; second, an acute form with intense and rapidly fatal septicaemia; and, third, a chronic form in which there is an intestinal infection, with toxic symptoms. The last form is very rare, but Plehn believes that infection occurs usually by the intestine, and some-:times through the gills if the fish is in poor cond-:ition. She does not consider the possibility of infection through a surface injury.

It is agreed by most workers that inoculation of <u>B. salmonicida</u> into the tissues of trout produces a condition//

condition similar to the natural disease.

Several cases have been recorded of disease pro-:duction in fish by Vibrios. Canestrini (1892-3) recorded a disease of eels caused by an organism like V. cholerae. This vibrio was pathogenic for fish and frogs, but not for warm-blooded animals. It re-:tained its viability longer in salt than in fresh water.

Bergmann (1909, 1912) described a disease of eels caused by a vibrio, and also a pathological con-:dition occurring in codling, with a similar cause.

The disease of eels took the form of tumour-like lesions. In the codling there was a condition of keratomalacia, i.e., destruction of the eyes, beginn-:ing with opacity of the cornea. The vibrio was small, actively motile, and frequently formed chains. It grew at 20°C. and up to 40°C. and was a facultat-:ive anaerobe. A pellicle was formed on bouillon and peptone water; acid and clot in litmus milk; glucose, lactose, saccharose, and maltose were fer-:mented with production of acid but not gas; the nitroso-indol reaction was negative. The organism was pathogenic for certain cold-blooded animals, such as eels, crabs, and roach, but not for carp or warm-blooded animals.

David (1927) recorded the occurrence of disease in carp caused by a vibrio. The organism was short, thick, motile by a single polar flagellum; optimum temperature/

temperature was 8°C. to 20°C. and when grown at 37°C. viability was not lost, but peculiar forms were pro-:duced. Growth on agar became more luxuriant on sub-culturing, and was yellowish. Gelatin was liquefied with formation of a bubble of gas at the top of the funnel of liquefaction. There was abundant growth on potato. Haemolysis took place on sheep-blood-agar after a few days. Milk was not clotted unless the strain had been repeatedly subcultured on agar and milk. There was poor growth on bouillon and peptone water, and the nitroso-indol reaction was negative in ordinary circumstances. Fluorescence or phosphorescence never occurred.

The disease was highly infectious. All fish were removed from the pond where it occurred, and when it was later restocked, the new stock became infected and rapidly died out.

Carp were the fish attacked, and pike and tench were sometimes affected. Roach, perch and trout appeared to be immune. There had at one time been many frogs in the neighbourhood of the pond, and these had disappeared, which led to the suggestion that they had been attacked and destroyed by the dis-:ease. Frogs could be killed by experimental in-:oculation of the vibrio into the dorsal lymph sac, Warm-blooded animals were not usually attacked, but one strain was found to cause some symptoms of dis-:ease in rabbits. The strain which was recovered

from/

from the rabbits could not be grown successfully on culture media.

The disease in nature took the form of a blood infection; there was gastro-enteritis, blood-stained slimy discharge from the cloaca, and often the appear-:ance of hæmorrhages on the skin.

Acid-fast bacteria producing lesions like those of Tuberculosis have been found in many cold-blooded animals, but the disease produced is not usually severe. Saprophytic acid-fast bacteria occur in nature, and Calmette (1923) considered that these on occasion become pathogenic for cold-blooded animals. Many attempts have been made to infect fish with mammalian tubercle bacillus, and vice versa, but with no satisfactory results. Calmette gave an account of the occurrence of tubercle-like disease in Amphibia, Reptiles, and Fishes, and referred to work done on the subject generally.

Johnstone and Moore Alexander (1913) described tuberculous lesions in a cod, from which an acid-fast bacillus and a Gram-positive coccus were isolated. The organisms were symbiotic, and the bacillus could not be grown apart from the coccus.

The bacterial flora of the intestine of fishes has received some attention, particularly in refer-:ence to the presence or absence of B. coli. It is generally accepted that B. coli is of very much less frequent occurrence in cold-blooded animals than in mammals./

mammals. Its presence in the intestine of fish appears to depend on the degree of pollution of the water. Brown (1917) found B. coli present in 39·8 per cent. of fish (Stenomus chrysops) examined, and in 10·8 per cent. of these B. welchii was also pres-:ent. The latter organism was found without B. coli in 19·3 per cent. of cases.

Houston (1903) found B. coli in 13 per cent. of sea fish, and stated that the organism was rare in fish in the absence of sewage.

Whipple (1904) found no B. coli in trout, perch or sun-fish in unpolluted water, and Johnson (1904) isolated B. coli from the intestines of 47 out of 67 fish in polluted water.

Bettencourt and Borges(1908) found only two strains of typical B. coli in 17 fish, reptiles and amphibia.

The results of other investigators are similar.

Anderson (1907) appears to have taken it for granted that B. coli is normally present in the in-:testine of fish, for he stated that in decomposition of fish, that organism multiplied, and along with autolysis, was responsible for destruction of the intestinal wall.

Hunter (1920-22) found that the muscle of freshly-caught fish was sterile, and the alimentary canal only contained bacteria if food was present. The mouth and gills contained organisms, and the body of the/

the body of the fish was invaded by these within 96 hours of death. The organisms which were found in decomposing salmon corresponded with those found in the water from which the fish were obtained. Hunter isolated a large number of types of bacteria, few of which had been described before. He studied particu-:larly those which took an active part in decomposit-:ion, as tested by their production of indol or foul odours. The majority of the organisms isolated were non-fermenting soil and water forms, and these chiefly responsible for decomposition were found to be B. fluorescens, B. cloacæ, and a bacillus which produced flesh-coloured colonies on nutrient agar. Very few sporing organisms were found, and no obligate anærobes.

Fellers (1926) found that bacteria were present only in the mouth, gills, and slime of live salmon, and these were the cause of decomposition. The gills spoiled first, then the tissue around the cloaca, while the back muscles remained fresh longest.

31 per cent. cocci, 4·5 per cent. sporing aerobes, 17·5 per cent. chromogenic aerobes, 36·5 per cent. non-chromogenic, non-sporing aerobes, 6·8 per cent. yeasts, 2 per cent. obligate anaerobes, and 1·7 per cent. spirilla were isolated.

Cocci were very active in the initial stages of decomposition, and sporing bacilli and yeasts were also present then. Later B. lactis aerogenes, B. coli, and Fluorescens types increased and outgrew

the others. River and sea water contained the same types of organisms as were found in the fish.

II. FURUNCULOSIS OF THE SALMONIDAE.

In 1926 and 1927 salmon in several Scottish rivers were observed to be dying from a disease re-:sembling Furunculosis. It was believed that a simi-:lar disease had occurred in previous years, but was not of sufficient frequency to demand investigation. It seemed that the disease was increasing in preval-:ence, and by 1927 it was anticipated that the cond-:ition might assume serious proportions; but the cold, wet weather of that year appeared to check it, for, as was stated above, Furunculosis is favoured by hot and dry weather, when the fish are overcrowded in low and impure water.

In the summer and autumn of 1926 several salmon which had died from a disease like Furunculosis were submitted for examination to the University Bacteriol-:ogy Department by Mr. Calderwood, of the Fishery Board. Some of the fish were in an advanced state of decomposition, and, although the general appear-:ance was suggestive of Furunculosis, the material was not suitable for bacteriological study.

Two salmon - one from the Kirkcudbright Dee, and the other from the Lyon, a tributary of the Tay, were fully examined, and an organism, corresponding in characters to B. salmonicida (Emmerich and Weibel), was isolated from heart blood, muscle lesions, liver and kidneys of both fish.

Two/

Two other salmon, from the Grimersta (Lewis), were also examined, but B. salmonicida was not found, nor was the pathological condition like that of Furuncul-:osis.

In 1927 an outbreak of Furunculosis was reported in the Kirkcudbright Dee, and two salmon were submitt-:ed for examination in June and July. One showed typical furunculous lesions (Plate I. Figs.1. & 2.) B. salmonicida was found in the heart blood, muscle les-:ions, peritoneal fluid, and kidneys. The other fish had a discoloured area near the cloaca, and the under-:lying tissue was dark and softened; but B. salmon-:icida was not present, and the condition appeared to be due to injury.

Furunculosis was also reported in the Lyon and Garry in Perthshire, but no specimens were obtained from this source.

In August two salmon from the Firth of Forth which had skin lesions were examined for Furunculosis, but B. salmonicida was not found. The lesions were of the nature of chronic ulcers, and may have been caused by injury followed by infection with water bacteria. The general condition of the fish was good.

Part of a salmon showing a skin lesion of the nature of an ulcer was sent from the Tweed, but B. salmonicida was not present in the specimen.

Four cases of ulcerative skin disease in salmon from/

from Banffshire were examined in the early summer, and B. salmonicida was not present in any case. Hume Patterson (1903) described a pathological condition in salmon which he claimed was caused by B. salmonis pestis, and this ulcerative condition seemed to be similar to the disease described by him. No organism resembling B. salmonis pestis, however, was found in these specimens. Several strains of bacteria were isolated from the ulcers, but these proved to be ord-:inary water bacteria.

A trout, Salmo truttae was received from an aquarium in September, and presented a typical pict-:ure of Furunculosis. B. salmonicida was obtained in almost pure culture from the heart blood. This fish was one of several which had been obtained from a tributary of the Tweed for an aquarium, and another of the same batch of fish was reported to have died from Furunculosis about a fortnight earlier. No further cases have occurred in the tank. As far as is known, there have been no cases of Furunculosis in the Tweed.

Eight dead trout were sent in September from a hatchery where Furunculosis was said to have occurred earlier in the season. Two of the fish were from a pond where cases had occurred, two from a pond where the presence of the disease had been suspected, and the remaining four from other ponds. The first six were Salmo fario, and the last two Salmo irideus.

No lesions were found in any of the fish, and the blood and viscera were sterile, except the intestines, the bacterial flora of which corresponded to that of the water in which the fish lived.

Ten living trout, all Salmo fario, were obtained from the same hatchery in October, and kept under observation in well-aerated tanks. Eight were two years old and two were four years. The two larger fish were killed after a few weeks, and examination for B. salmonicida yielded negative results. Three of the young fish died in two to eight weeks after being received. Two showed no sign of disease, and death was probably due to injury or unnatural conditions, while the third showed degenerative changes in the fins and skin. B. salmonicida was not found. The remaining trout appeared quite healthy, and were used for experiments, referred to later.

Water snails and water from the same hatchery were also examined, but B. salmonicida was not present. The heavy floods which had occurred in the later part of the season would of course render any such organisms relatively scanty, if present.

SPECIAL INVESTIGATION OF CONDITIONS IN THE KIRKCUDBRIGHT DEE.

Outbreaks of Furunculosis having been reported in 1926 and 1927 among salmon and trout in the Kirkcudbright Dee, special attention was given to conditions/

conditions in this river. In 1927 the disease was noticed there towards the beginning of July, and the specimens mentioned above were submitted for examin-:ation. Owing to excessive rains the river became flooded, and no more dead or diseased fish were found during the rest of the season.

When the floods had subsided somewhat, at the end of August, a visit was made to the district. No fish were being caught owing to the high water, but the general conditions were investigated. The Dee is fairly free from sewage or other contamination almost as far as the tidal area. Just above that region, a few farm drains enter the river, and bear a considerable amount of organic matter and sewage. The effect of the sewage on the viability of B. salmon-:icida was tested; the organism was found to die out very rapidly and could not be recovered after 24 hours. The same result was obtained with a specimen of very highly polluted water entering the river within the tidal area.

The types of bacteria found in the river water, and in the drains flowing into it, were ascertained, and were the usual organisms of water and sewage with the addition of some soil bacteria which had been washed in by the floods. B. salmonicida was not found.

Some of the farm drains contained a large number of/

of coliform bacilli, but the amount of sewage discharged into the river was not enough to contaminate it seriously, except in a large pool at the head of the tidal area, where the water accumulated at high tide, and into which polluted water from the lower part of the river was also carried by the tide.

B. fluorescens, an organism very frequently isolated from dead salmon, and which has been found by the writer to be pathogenic for cold-blooded animals under experimental conditions, was present in large numbers in all samples of the water.

Useful information regarding the parts where dead and diseased fish were most frequently found was obtained. The highly contaminated pool referred to above was said to be the place where it was most common to find these fish, and young sea trout were described as swimming wildly about in the pool, and then dying in a condition believed to be Furunculosis.

Although B. salmonicida was not found in the affluents of the river, nor was it viable in water from them for any length of time, yet the presence of such bacterial and organic contamination may possibly be prejudicial to the health of fish, especially those just entering fresh water from the sea.

PATHOLOGY

The disease Furunculosis of the Salmonidae was found to be a general infection. The causative organism, B. salmonicida, appears in large numbers in the blood of the affected fish, and lesions are developed, especially in parts of the body where there is a capillary network. Thus the typical lesions are subcutaneous; the highly vascular con-:nective tissue of the dermis is entirely destroyed in parts, and this results in the formation of the so-called "pus", which is a mass of necrotic tissue. There is considerable destruction of the capillaries, and blood cells are set free among the tissue fibres. The underlying muscle fibres are destroyed, and the process continues in the connective tissue between the muscle bundles. Reddish granular material is found in the lesions, and consists of necrotic muscle fibres, free blood corpuscles, and large numbers of bacteria. There is complete absence of tissue reaction, and no leucocytosis.

The kidney is destroyed in a similar manner. As is shown in Plate II., Fig. (1), the capillaries of the glomeruli are broken down, and similar vascular lesions appear in other parts of the organ. The whole kidney becomes a semi-fluid necrotic mass. The spleen is reduced to the appearance of a loose blood clot, as shown in Plate IV., Fig. (2), there being no structure left in the organ/

organ, which is seen to consist of free cells and num-:erous bacteria.

The organs generally are congested, the intestine in most cases especially towards the cloaca, and the liver shows numerous minute hæmorrhages. Sections of tissues examined microscopically show bacilli pres-:ent in and around blood vessels, the walls of which are often damaged with effusion of blood into the surrounding tissue.

BACTERIOLOGICAL EXAMINATION.

Technique.- An extract of fish flesh was the basis of the culture media used. The extract was prepared from cod flesh, which was cut up, put in the required amount of distilled water, slowly heated to 100°C., boiled until the flesh was completely broken up, allowed to cool, and filtered. One pound of cod flesh was extracted in one litre of water. This ex-:tract was used for making nutrient bouillon, agar and gelatin media. The optimum reaction for growth of the organisms studied was found to be P_H 7·6—7·8. Otherwise the culture media used were the same as in general bacteriological work.

The bacteria isolated from fish grew also on ordinary meat-extract media, but more profuse growth was obtained on fish-extract media.

The optimum temperature for growth of these org-:anisms/

organisms was found to be 15°–20°C. as a rule.

Examination of specimens was carried out with all aseptic precautions. The animal was washed with dilute formalin or lysol, then with methylated spirit, which was allowed to dry or was burned off. The part in which an incision was to be made was seared with a copper spatula, and all instruments used were steri-:lised by boiling.

Cultures were made from all organs and lesions on fish-extract-agar plates, and incubated for 48 hours at room temperature. For microscopical examin-:ation smears were made and stained by Gram's stain or by other appropriate methods. Tissues were fixed for histological examination.

Four strains of B. salmonicida were isolated and studied. These were obtained from:

(1) Salmon from Kirkcudbright Dee
(2) Salmon from Tay
(3) Salmon from Kirkcudbright Dee
(4) Trout from aquarium.

Other organisms isolated from heart blood and tissues of diseased fish were of the following types:-

(1) Bacillus fluorescens.— Organisms of this group were found to be pathogenic when introduced into the tissues of cold-blooded animals such as frogs. Further experiments are being carried out to test their pathogenicity for trout, and this subject will be dealt with in a later communication.

(2) Non-chromogenic, Gram-negative bacilli which/

which are non-motile or motile (with polar flagella).- These organisms probably belong to the Achromobacter group, members of which are common in water.

(3) Chromogenic bacilli of the Flavobacterium group.- These were non-pathogenic for frogs.

(4) Cocci and Micrococci - Non-pathogenic for frogs.

(5) Several types of Yeasts.

(6) A Vibrio, which appears to be highly patho-:genic for trout. This is being investigated further.

As well as the strains of B. salmonicida above mentioned, four strains were obtained from the Lister Institute, London, and were compared with the others.

CHARACTERS OF B. SALMONICIDA

The cultural and biological characters of the strains of B. salmonicida was found to be a short rod-shaped organism 1-4μ x 0·8-1μ, with a tendency to occur in pairs. All strains were non-motile and non-sporing.

In staining reactions, the bacillus was Gram-negative and tended to stain unevenly, especially in the tissues, where the short forms often showed bi-:polar staining.

The organism was an aerobe and facultative anaerobe.

The temperature range was found to be from 5°C. to/

to 32°C., and the optimum temperature for growth was 20°C. Growth occurred on all ordinary media, but better on fish-extract-agar than on meat-extract-agar; abundant growth occurred on media containing rabbit's blood and serum. The optimum reaction was P_H 7·6–7·8.

Agar plate culture.– In 24 hours, at 15°C–20°C., a delicate growth of small transparent colonies was seen, and in seven days these were on an average 1 mm. in diameter, circular, raised, slightly brownish in colour, semi-opaque, moist and glistening. Under aerobic conditions the medium became brown, due to a diffusible pigment formed by the organism. Chromo-:genesis was at a maximum at the above temperature, and on a medium with an initial P_H of 7·8. The pigment began to appear in four to six days, and then increased until the medium was of a deep coffee col-:our.

Agar stroke culture.– There was a moist whitish line of growth, slightly granular, in 24 hours, and in about a week this became brownish. The diffusible brown pigment was produced as on the plate culture.

Gelatin stab culture.– Liquefaction began in about 24 hours, and it was noticed that the gelatin was generally first liquefied at the foot of the stab and the medium liquefied progressively from below up-:wards. The growth formed a mass at the foot of the funnel of liquefaction, with flocculi in the lique-:fied/

liquefied medium above. Strain 4 formed small quantities of gas as well as liquefying the gelatin, but no gas formation was seen in the cases of the other strains. When the organism was allowed to con-:tinue growing in the liquefied gelatin for six weeks to two months, the upper part of the medium became brown.

Bouillon culture.— The growth formed a deposit at the foot of the tube, with flocculi throughout the liquid, but no pellicle. Slight pigment was produc-:ed in about two months, especially if the culture was contained in a small flask, where a greater sur-:face was presented to the air than in tube cultures.

There was poor growth on peptone water, and no indol production. Strain 4 grew better in this med-:ium than the other strains.

Sugar reactions.— All strains of B. salmonicida tested fermented glucose and mannite in about 24 hours, with production of acid, and strains (3) and (4) also produced gas in two to three days. In mannite, gas production was much greater than in glucose. Sacch-:arose, dulcite and lactose were not fermented, al-:though abundant growth occurred; the medium became alkaline, and in a few days pigment was produced.

Strains one and two fermented starch and glycerol with production of acid; maltose with production of both acid and gas; while inosite, adonite, rhamnose, xylose, raffinose, and arabinose were not fermented.

Growth/

Growth on serum-agar was exceptionally abundant, with intense pigment production, the medium becoming almost black.

On blood-agar growth was also abundant, and hæm-:olysis rapidly took place.

Loeffler's solidified serum was slowly liquefied by all strains of the organism and the medium became brown.

Litmus milk was acidified and slowly peptonised without coagulation.

On potato there was rather slow growth, brownish, slightly rough and raised.

Anaerobic growth.- The organism was found to grow well on Henry's plates and in Buchner tubes, but no pigment was produced. There was anaerobic growth in Robertson's bullock-heart medium under liquid paraffin, and in a similar medium prepared from cod's heart, the flesh was reddened.

Pigment production.- Since the brown pigment of B. salmonicida has been regarded as of so much import-:ance, its production was studied carefully. It was found to be produced only under aerobic conditions, and on media which were not too acid. When the react-:ion was more acid than P_H 7·6 pigment was proportion-:ally less, and at higher and lower temperatures than the optimum for growth, chromogenesis was also re-:strained. The formation of pigment on the various media was noted, and it was found to be at a maximum on/

on solid media such as fish-extract-agar and serum-agar, but pigment also appeared on fluid media though after a longer time. In one to two months in bouill-:on and liquefied gelatin the fluid towards the sur-:face, where free oxygen was present, became brownish. Solutions of carbohydrates in peptone water became pigmented when B. salmonicida had been growing in them for a few days, provided there had not been acid fermentation. Pigment was not produced in peptone water.

An experiment was carried out to show that the brown pigment is a product of aerobic growth, both active growth and free oxygen being necessary. Three cultures of the organism were put up at the same time on fish-extract-agar slopes. All were incubated at room temperature: (1) aerobically; (2) and (3) anaerobically in Buchner tubes. In four days pig-:ment began to appear in (1), and all were kept for 14 days, when there was intense pigmentation in (1) and no trace of pigment in (2) or (3). Then (2) was removed from the Buchner tube and left exposed to air, while (3), on removal from the Buchner tube, was sterilised by exposure to 60°C. for one hour. On the same day a fresh culture (4) was made and kept under aerobic conditions. Four days later (2) and (4) showed commencing pigment production, and in six days this was marked; (3) remained unpigmented.

Thus,/

Thus, pigment is produced by B. salmonicida when actively growing in the presence of air, and is not the result of exposure of the products of growth to air.

RELATIONSHIPS OF B. SALMONICIDA

The characters of this organism are such as to render its attachment to any known group difficult. The name was probably given with the intention of de-:scribing the organism in terms of its pathological activities, but it tends to suggest affinities with the Pasteurella group, while the only resemblances are the appearance of bipolar staining in the tissues, and the general nature of the disease. Organisms of the Pasteurella group do not liquefy gelatin, are non-chromogenic, and also do not ferment sugars with pro-:duction of gas.

The production of a brown pigment is a difficulty in allocating B. salmonicida to any group. The genus Flavobacterium (Bergey et al.) is characterised by production of yellow to orange pigment, and no true brown pigment is formed by members of this group, nor do they ferment sugars with production of gas. One of the group, B. brevis (Frankland) shows bipolar staining, but in no other way resembles B. salmonicida. The diffusible nature of the pigment is also a point of difference, for the Flavobacteria produce colonies themselves/

themselves pigmented, and in the case of B. salmoni-:cida the colonies are not distinctly pigmented but the pigment is a product of growth and appears in the medium.

The genus Pseudomonas produces diffusible pig-:ment, but this is green, blue or yellowish-green. Sometimes strains of B. fluorescens produce greenish pigment which becomes brownish in old cultures, after the bacilli have themselves ceased to multiply, but the pigment of B. salmonicida is essentially a pro-:duct of active aerobic growth.

A coccus has been met with which produces brown diffusible pigment, and agar slope cultures of this organism are very similar to those of B. salmonicida.

If the organism is to retain its present name, it must be amended to B. salmonicidus, for grammatical reasons.

EXPERIMENTS ON PATHOGENESIS OF B. SALMONICIDA.

The animals used for these experiments were frogs, goldfish, minnows, trout, guinea-pigs, mice, and one sea fish, Zoarces viviparus.

The methods used were: subcutaneous, intraperi-:toneal, or intramuscular injection of emulsion of young cultures of B. salmonicida in sterile 0·6 per cent. saline; application of cultures to scarified areas of skin; exposure of animals to infected water; and feeding with cultures.

Frogs.— Frogs were kept in jars with a small amount of water.

(1) 1/5 agar slope culture of B. salmonicida strain (1), injected into dorsal lymph sac. Death occurred in four days from profound general bacteraem-:ia, with considerable œdema and multiple small hæm-:orrhages. B. salmonicida was recovered in pure culture from blood, liver, lung, kidney, brain and œdematous fluid.

(2) As (1), but more dilute emulsion (opacity of emulsion equal to Brown's opacity Standard No. 6). Marked œdema appeared and ulcerating lesions formed under the skin. The frog was killed after 26 days, and B. salmonicida and B. fluorescens were found in the heart blood.

(3) The animal was placed in water infected by B. salmonicida, strain (2). Numerous superficial lesions/

lesions developed and the frog died in 42 days. Small numbers of B. salmonicida were recovered from lesions and heart blood, but many other organisms were present, including B. fluorescens. B. salmon-:icida could not be said to be the cause of death.

(4) Frog was inoculated in the muscle of hind leg with 0·5 c.c. of same emulsion of B. salmonicida as used in experiment (2). The leg became swollen, but the animal recovered completely.

(5) Animal fed with cultures of B. salmonicida, strain (1), for one month, but infection did not occur. The animal died eventually from a subsequent infect-:ion by an organism which resembled Bacillus hydro-:philus fuscus (Emerson and Norris, 1903).

(6) One c.c. of filtered bouillon culture of B. salmonicida, strain (1)(grown for one month), in-:jected intraperitoneally. The result was negative.

(7) Inoculated intraperitoneally with 1/10 agar slope culture, B. salmonicida, strain (1). The frog died in four days, and B. salmonicida was recovered from all organs.

(8) Inoculated as (7) with B. salmonicida, strain (2). Died in three days, and the bacillus was recovered from all organs.

(9) Inoculated as (7) with B. salmonicida (Lister Institute strain). Result was negative. The strain may have lost virulence by repeated cultur-:ing on artificial media.

(10)/

(10) Frog inoculated with B. salmonicida (Kennet strain). Result negative.

(11) Inoculated with Cocquet strain. Died in one month, and B. salmonicida was recovered from heart blood.

(12) Inoculated with Lledr strain. Died in 14 days, and B. salmonicida recovered in almost pure culture from heart blood.

(13) Frog was placed in the same tank with (11), which had developed external lesions, and after (11) had died, cultures of B. salmonicida (Cocquet strain) were added at intervals to the water. No infection took place.

(14) Inoculated intraperitoneally with 1/5 agar slope culture, B. salmonicida, strain (2). Died in two days, and B. salmonicida recovered in pure culture from heart blood.

(15) Inoculated with 1/7 agar slope culture, B. salmonicida, strain (2), in dorsal lymph sac. Killed after one day, and B. salmonicida recovered in pure culture from heart blood.

(16) Inoculated as (15). Killed after two days, and result as in (15).

(17) Inoculated as (15). Died in two days, and result as in (15).

(18) Inoculated as (15). Died in five days, and result as in(15).

(19) Inoculated subcutaneously with 1/5 agar slope/

slope culture, B. salmonicida, strain (3). Died in nine days, and B. salmonicida recovered in pure culture from heart blood.

(20) Inoculated as (19), with B. salmonicida, strain (4). Died in six days, and B. salmonicida recovered in pure culture from heart blood.

(21) Inoculated intraperitoneally with 1 c.c. (½ agar slope culture), B. salmonicida, strain (1), killed culture. The result was negative.

Goldfish.- (1) Water infected, B. salmonicida, strain (1). Died in 16 days, and B. fluorescens found in pure culture in heart blood. No sign of infection by B. salmonicida.

(2) Water infected as (1). Died in 20 days, and B. salmonicida and B. fluorescens found in heart blood.

(3) B. salmonicida, strain (2), rubbed into scarified area of skin. A discoloured, softened lesion was formed at the site of inoculation, and the fish died in seven days. B. salmonicida and a few colonies of B. fluorescens were grown from heart blood.

(4) Inoculated as (3). Died in eight days, and result was the same.

(5) Uninjured fish put beside (3) and (4). Died in 25 days, and B. salmonicida and B. fluorescens found in heart blood.

(6) Loopfuls of B. salmonicida, strain (1), were introduced/

introduced into mouth of fish. Died from injury.

(7) Cultures put in mouth as in (6). Died in seven days, and B. salmonicida and B. fluorescens found in heart blood.

Minnows.- (1-5) Kept in water infected with B. salmonicida, strain (1). Remained healthy. Subsequently fed with cultures of B. salmonicida. (Fish readily took loopfuls of culture when hungry). Infection did not take place.

(6) Inoculated with B. salmonicida, strain (1), by rubbing culture into scarified area of skin. At site of inoculation a lesion developed like the lesions in salmon. Died in five days, and B. salmonicida was recovered from heart blood, body fluid and deep muscle lesions.

Salt water fish.- Viviparous Blenny. 1/10 agar slope culture, B. salmonicida, strain (1), was injected intraperitoneally. The fish died in two days, but the ovarian sac had been injured in inoculation, and this may have been responsible for death. At autopsy this organ and the body fluid contained large numbers of B. salmonicida, which were viable in the salt water fish. The heart blood was sterile.

Trout.- S. fario, aged two years, was placed in natural water contaminated artificially with B. salmonicida, strain (2). Died in four days, and B. salmonicida/

B. salmonicida was recovered in small numbers from the heart blood. The fish may have been in poor condition after transportation to the laboratory, or may have received injury.

(2) As (1), but tap-water used. The fish re-:mained alive and healthy.

(3) S. fario inoculated with B. salmonicida, strain (1), by rubbing culture into scarified skin in front of adipose fin. Died in five days, and B. salmonicida recovered in pure culture from heart blood and deep muscle lesion. A lesion like that in natural infection had developed at the site of inoculation, and before dying, the fish appeared to lose power of orientation, and swam wildly about in a vertical position.

Warm-blooded animals.- Two guinea-pigs were inoculated subcutaneously with 1/5 agar slope cult-:ures, B. salmonicida, strains(1) and (2) respectively. A slight hard swelling appeared at the site of inocu-:lation in each case, but had disappeared in about two weeks.

One guinea-pig had 1/10 agar slope culture, B. salmonicida, injected intravenously. There were no effects.

One guinea-pig was injected subcutaneously with 1/10 agar slope culture, B. salmonicida, killed by exposure to 60°C. for one hour. No effects were noted.

Two/

Two mice were inoculated subcutaneously with B. salmonicida, strain (1), and small hard swellings appeared as in the guinea-pigs. One mouse was kill-:ed in eight days, and the swelling contained sterile pus. The other mouse recovered in about two weeks. Actual infection had not taken place. A local leuco-:cytosis had apparently occurred as a reaction to the inoculation.

B. salmonicida cannot multiply at 37°C. or sur-:vive for more than three or four days at this temper-:ature. It cannot, therefore, establish itself in the tissues of warm-blooded animals.

The above experiments prove that B. salmonicida is highly pathogenic for certain cold-blooded animals, when living cultures are introduced into the tissues.

When experimental animals were infected, the organism invaded the blood stream, where it multiplied and was carried all over the body. The walls of the blood vessels were injured, and petechial hæmorrhages appeared. The organs generally were congested; sections of these showed bacilli in and around the blood vessels, and areas of necrosis, where bacilli and blood cells were free among breaking-down tissue. This was especially the case in the kidneys, where the glomeruli were destroyed, and contained large masses of bacteria.

In the frog, considerable œdema was produced, when the infection ran a sub-acute course, and this was/

was probably due to (1) escape of plasma from the injured blood vessels, and (2) renal disease, with destruction of the glomeruli. Pathological changes also took place in the lungs of that animal, where the bacilli were to be seen blocking the vessels, and the surrounding tissues were degenerating.

Haemolysis occurred to some extent in the blood, and in frogs only occasionally was there any degree of phagocytosis by polymorphs or mononuclears. The amount of blood in the auricles of the frog at death was greatly diminished from that in a healthy frog, or one which had died from a different infection. In goldfish and minnows haemolytic changes were even more marked, and the blood appeared as a pale, thin fluid. Few red blood corpuscles remained intact, and the plasma contained many free and desintegrating nuclei.

There was no evidence that B. salmonicida produced toxins when grown on culture media. Intraperitoneal injection into frogs of large doses of old filtered bouillon cultures, and of killed cultures produced no effect.

Frogs and minnows could not be infected by exposure to infected water or by feeding. Goldfish were, however, infected by these methods, but B. fluorescens was found along with B. salmonicida in their tissues after death, and represented probably a concomitant or secondary infection. This organism was found by experimental/

experimental inoculation to be pathogenic to goldfish and frogs.

The experiments with trout have been somewhat in-:conclusive, and these fish were from a district where Furunculosis was said to have occurred. In the case of trout (3), however, there is little doubt that the experimental inoculation with B. salmonicida was the direct cause of death. The behaviour of this fish before death was interesting, as it was the same as had been described by many observers of fish dying from Furunculosis in nature.

In the experimental disease, as in Furunculosis in nature, the condition is essentially a general in-:fection or bacteræmia.

EXPERIMENTS TO TEST IF B. SALMONICIDA CAN INFECT TROUT OVA.

(1) Eyed ova of Salmo trutta were used.

(A) 18 ova were kept in a shallow dish in tap water which was changed daily.

(B) 18 ova kept as (1), and the water con-:taminated daily with B. salmonicida

(C) 30 ova kept in running tap water.

In ten days hatching was completed.

	A.	B.	C.
Died in egg	6	10	2
Died on hatching	6	4	0
Died subsequent to hatching ..	3	0	0
Living fry	1	0	28
Living ova removed	2	4	0
	18	18	30

Seven/

Seven ova were removed from B. at intervals, the surface sterilised by washing with 1/1000 Mercuric bichloride, or Absolute Alcohol, then with distilled water (sterile), and the contents plated on agar. Two ova from A. were similarly treated. B. salmonicida was not recovered from the ova.

It will be seen from this experiment that trout ova when kept in still water, although it is changed frequently, do not survive well. There was slightly greater mortality among the ova kept in water contaminated with B. salmonicida than among those kept in pure water, but there was no evidence that this was due to invasion of the ova by that organism.

(2) Ova of Salmo irideus in which the eyes were just formed were used, and they were kept in running tap water.

(A) 26 ova in pure water.

(B) 27 ova, in water to which B. salmonicida was added daily.

Hatching was complete in sixteen days.

	A.	B.
Died in egg	1	2
Died on hatching	4	2
Living fry	20	22
Removed alive	-(1 lost)	1
	26	27

Two ova from B. which had died, and one living, had/

had the surface sterilised as in experiment (1), and B. salmonicida was not found in cultures of the con-:tents.

In this experiment, the contamination of the water with B. salmonicida had no effect on the viabil-:ity of the ova.

It is highly improbable that healthy ova can be invaded by B. salmonicida.

THE QUESTION OF APPLYING AGGLUTINATION TESTS FOR THE EXACT IDENTIFICATION OF B. SALMONI-:CIDA STRAINS.

It has not been possible to perform agglutinat-:ion experiments with B. salmonicida as the bacillus was found to be auto-agglutinable in salt solutions of as low concentration as 0·1 per cent. NaCl. Even in distilled water, emulsions were unstable. Phos-:phate bouillon was used to emulsify cultures, but with no better result.

VIABILITY OF B. SALMONICIDA

Resistance to heat.- When agar slopes were in-:oculated with B. salmonicida and incubated at 37°C., no growth appeared; after three days at this temper-:ature, growth did not take place when the cultures were removed from the incubator and kept at room temperature, the organism having died out.

In/

In bouillon culture the organism was killed in one minute at 60°C.

Resistance to drying.- Cultures of B. salmon-:icida were smeared on sterile pieces of glass, and these were kept in a sterile Petri dish, where the smear dried. At intervals a piece of the glass was put in bouillon, and this was incubated at room temp-:erature. B. salmonicida was found to die out when dried for six hours.

Viability in water.- Emulsions of young agar slope cultures of B. salmonicida were made in various waters and plated out at intervals to determine the time during which the bacillus remained viable in the different specimens.

In all cases, when there were other bacteria present, it was difficult, if not impossible, to re-:cover B. salmonicida. This was mainly due to the fact that the latter organism grew slowly, and minute colonies only became visible on the agar in 48 hours, whereas other bacteria found in water, such as B. fluorescens and coliform organisms, etc., produced relatively large colonies in that time, and B. salmon-:icida was thus outgrown. This refers especially to surface plating. Further, the typical brown pigment was not apparent in mixed cultures with such organisms as/

as B. fluorescens. If the water was plated out by the shake method, it was difficult to distinguish the colonies of B. salmonicida growing under the surface of the medium, except by subculturing each, as they had no distinctive appearance, and did not produce pigment under conditions of reduced oxygen tension.

EXPERIMENTS ON VIABILITY OF B. SALMONICIDA IN WATER.

(1) A young agar slope culture was emulsified in the following unsterilised waters :-

1. Distilled.
2. Tap.
3. Lightly polluted natural water.
4. Sea.

Fifteen c.cs. of the waters were used, and emulsions of equal opacity were made in each. At intervals of 24 hours loopfuls of the emulsions were plated out. The results are shown in the table following:-

Days	Distilled Water	Tap Water	Natural Water	Sea Water
1	+	+	+	+
2	+	+ scanty	+ scanty	+ scanty
3	+	+ very scanty	0	0
4	+	0	0	0

+ Signifies growth.

(2) Sterilised waters were used: (1), (2) and (4)/

(4) were the same waters as in experiment (1), and (3) was heavily polluted natural water. Emulsions were made in 50 c.c. of water, and at intervals of 24 hours 1 c.c. and 0·1 c.c. were plated out by the shake method.

Days	Distilled water	Tap Water	Natural water.	Sea Water
1	+	+	+	+
2	+	+ scanty	+	+
3	+	+ very scanty	+	+ very scanty
4	+	0	+ scanty	0
5	+	0	+ very scanty	0

+ Signifies growth.

(3) Water from the Kirkcudbright Dee, and from a highly polluted drain entering it, were used. Emulsions were made in 15 c.c. of water.

(a) Unsterilised waters-

Days	Dee Water	Drain Water
1	+ scanty	0
2	0	0

(b) Sterilised waters-

Days	Dee Water	Drain Water
1	+	+
2	+	+
3	+	+ scanty
4	+ scanty	0
5	+ very scanty	0

Arkwright/

Arkwright states that he could not recover B. salmonicida from sea water after 19 hours, but this may have been due to the fact that rapid agglutination occurs in that water, and the clumped organisms rapid-:ly sediment in the tubes, so that cultivation from the supernatant fluid yields negative results.

It will be noticed that B. salmonicida dies out more rapidly in ordinary tap water than would be ex-:pected. This is being further investigated, and samples of water from different places tested.

Plehn states that B. salmonicida dies out rapidly in pure waters and remains viable longer in the pres-:ence of organic pollution, and she also thinks that the bacillus may persist for a long time in mud.

It appears unlikely that B. salmonicida exists as a saprophyte in water. It is to be noted that epizootics of Furunculosis in Scotland have not occurr-:ed in the more polluted salmon rivers, but in a rela-:tively unpolluted river, the Kirkcudbright Dee, and in an exceptionally pure river, the Lyon. This seems to suggest that the bacillus is introduced in some way, as by importation of fish carrying the disease.

In the case of an epizootic, B. salmonicida is probably liberated from diseased fish and distributed throughout the water. It can apparently survive for a period sufficiently long to allow of its widespread dissemination. Plehn found large numbers of the bacillus in river water in which the disease was pre-:valent./

prevalent.

The subject of sources and modes of infection requires further study.

CONCLUSIONS

1. The disease which occurred among Salmonidæ in the Kirkcudbright Dee and the Lyon, in the summers of 1926 and 1927, corresponds to the so-called "Furun-:culosis of the Salmonidæ", as described in other countries. Trout which had been obtained from the Tweed in 1927, and placed in an aquarium, were also found to be infected with Furunculosis.

2. A bacillus agreeing in characters with B. salmonicida (Emmerich and Weibel) was isolated from four diseased fish, two salmon from the Kirkcudbright Dee, one salmon from the Lyon, and one trout from an aquarium. This organism was proved pathogenic under experimental conditions for certain cold-blooded ani-:mals (frogs, minnows, goldfish and trout).

3. The bacillus is not pathogenic for warm-blooded animals.

4. The disease is essentially a general infect-:ion.

5. The so-called furunculous lesions are areas or foci of subcutaneous necrosis, involving also the underlying muscle. B. salmonicida is present in large numbers in these foci, which show no tissue reaction/

reaction comparable to an inflammatory condition as seen in certain bacterial infections of warm-blooded animals. The so-called "pus" in these lesions consists entirely of semi-fluid necrotic tissue with a certain amount of blood admixture. The specific organism is present in large numbers in this material.

6. The disease is apparently less prevalent in cold, wet summers than in warm dry weather. The occurrence of floods during a season in which there has been an outbreak of Furunculosis causes apparent disappearance of the disease. This may be due to the washing away of dead and diseased fish or to actual amelioration of the conditions of the river.

7. No connection between pollution of rivers and occurrence of Furunculosis has been found.

III. BACTERIAL FLORA OF SPOILAGE IN FISH; AND POTENTIAL PATHOGENESIS OF CERTAIN WATER BACTERIA.

The salmon examined were received from all parts of Scotland, and frequently there was a lapse of sev-:eral days between death and autopsy. This loss of time, and possibly contamination by handling, was the cause of considerable spoilage of the fish. The salmon were examined primarily with a view to deter-:mining the presence or absence of B. salmonicida, the causative organism of Furunculosis, and that bacillus was isolated from certain of the fish. As might be expected in the circumstances, numerous bacteria associated with spoilage were also isolated. Some at least of these organisms appeared to have been derived from water; some strains were simple contaminants, while others took an active part in spoilage, but certain strains were found to be patho-:genic for frogs and fish under experimental condit-:ions.

Work with experimental animals suggested that, although the presence of many of the organisms was due to post-mortem invasion of the tissues and ordin-:ary spoilage, some represented ante-mortem or second-:ary infections. Experimental animals were always autopsied/

autopsied immediately after death, and in the case of frogs, frequently while the heart was still re-:sponsive to stimulation. Thus no time was given for post-mortem infection to occur, but yet when cultures were made from the heart blood, organisms other than the strain used in inoculating the animal were found to be present. These organisms repres-:ented a secondary or terminal infection.

Several cases of death of uninoculated animals, kept as controls or stock, occurred, and examination immediately after death showed organisms to be present in the heart blood. Strains of B. fluorescens and organism (G.F.C.) were isolated from the heart blood of two uninoculated goldfish which died. B. fluor-:escens was found to be pathogenic for frogs, and the other organism for minnows, but not for frogs. The goldfish had not been injured in any way, and death was apparently due to infection by either or both of the bacilli found in the blood. Two gold - fish were kept in water contaminated with B. salmon-:icida; one died without infection by that organism having taken place, and a pure culture of B. fluores-:cens was isolated from the heart blood. Infection by B. salmonicida occurred in the other fish, but B. fluorescens as well as this organism was found in the heart blood. Two gold fish were fed with loopfuls of B. salmonicida; both died, and the heart blood of one gave a mixed culture of B. salmonicida and B. fluorescens,/

fluorescens, while that of the other yielded B. fluor-:escens in pure culture. These observations show that B. fluorescens, a common water organism, can infect goldfish under certain conditions.

Accidental infection of frogs was also found to take place. One frog was fed with B. salmonicida at intervals of a few days, and kept under observat-:ion for some months after experimental feeding was stopped. The animal eventually developed ulcerous lesions on the lower surface and then died. A pure culture of a bacillus (F. d7M.) was isolated from the heart blood, and proved highly pathogenic for frogs, in which it produced a general infection. Another strain of a bacillus the same as this was recovered from the heart blood of a frog, which died subsequent-:ly to inoculation with an organism of the Flavobact-:erium group which was non-pathogenic. A strain of B. fluorescens was also isolated from this case. Two strains of bacilli very similar to the preceding (F.d7M. etc.) were isolated from the heart blood of frogs, one of which died in consequence of inoculation with a strain of B. fluorescens, and the other accid-:ently after inoculation with a strain of B. salmoni-:cida which proved a-virulent. B. fluorescens was also present in the latter case. These two strains (F. d29J. & F. Ken.) were, however, not pathogenic for frogs by inoculation. They were present in the blood of frogs along with pathogenic organisms in both/

both cases, and probably represented ante-mortem in-:fections, while not actually pathogenic.

Three stock trout (Salmo truttae) died without experimental interference, and from the heart blood of each, a strain of a bacillus (T.C. d280 H.B.1 etc.) was isolated. These were strains of the same organ-:ism and all were highly pathogenic for frogs. From the heart blood of two of the trout, a bacillus of the Flavobacterium group was isolated. This organism was not pathogenic for frogs. One of the trout died during a week-end and so was not examined immediately after death. The heart blood contained, in addition to the pathogenic bacillus and the Flavobacterium organism referred to above, a strain of B. fluorescens and an organism of the Chromobacterium group. In this case, post-mortem infection had probably taken place. The heart blood of the third trout yielded a culture of an atypical coliform bacillus, as well as the pathogenic organism. This coliform organism may have been derived from the intestine of the fish.

The organisms isolated represented : (1) primary infection, e.g., actual cause of death; (2) secondary infection; (3) post-mortem infection; (4) surface contaminants. B. salmonicida, in the case of certain fish which died under natural condit-:ions, belonged to the first class: B. fluorescens, "F. d7M." etc., and "T.C. d280. H.B.1" etc., isolated from experimental animals, also appear to belong to this/

this class. B. fluorescens, also on occasion belongs to the second class, and the other organisms isolated from experimental animals probably do as well. Pig-:ment producing organisms other than B. fluorescens, non-pathogenic non-pigment producing bacilli, and probably also cocci, belong to the group causing post-mortem infection. Many of the latter group and some of group (2) probably take an active part in spoilage of fish. Cocci are often probably merely surface contaminants.

Fellers (1926) found that cocci were numerous in the early stages of spoilage, but were later overgrown. He found that the non-sporing, non-chromogenic group were most active in decomposition of salmon, while members of the Flavobacterium group were also active in spoilage. B. fluorescens was very active and numerous.

Over sixty strains of bacteria were isolated from the following sources :

3 furunculous salmon
4 salmon dead from unknown causes
1 salmon kelt
1 furunculous trout
3 trout which died in aquarium
4 experimental frogs
6 goldfish.

The types of bacteria isolated were similar to those found in water. The following types of organ-:isms were met with :

Micro-cocci/

Micro-cocci. Five strains; three resembling Micro-coccus candicans, from surface lesions on salmon and intestine of trout; one, unidentified Gram-nega-:tive, from furunculous lesion in salmon; and one strain of Micro-coccus luteus, from gills of trout. None of these cocci were pathogenic for frogs. They were apparently surface contaminants, derived from water.

Flavobacterium. Five strains; one unidentified, from furunculous lesion of salmon; one, resembling Flavobacterium sulphureum except in shade of pigment, from liver of salmon; and three, resembling Flavo-:bacterium arborescens, from heart blood of two trout which died in aquarium, and stomach of frog. None were pathogenic for frogs, but they may have taken some part in spoilage.

Serratia. One strain of Serratia marescens from heart blood of salmon kelt, which was in a state of advanced decomposition.

Chromobacterium. Two similar atypical strains of Chromobacterium violaceus, from heart blood of a trout and skin lesion of a salmon kelt. None of these pigment producers were pathogenic, and they were apparently derived from water.

Pseudomonas. More than twenty strains of Pseudomonas fluorescens were isolated from heart blood and lesions of salmon and experimental animals. All strains of this organism tested were pathogenic for frogs./

frogs.

Achromobacter. Seventeen strains of organisms which agreed in general characters with the group Achromobacter were isolated from heart blood and lesions of salmon and experimental animals. One isolated from liver of salmon was identified as Achromobacter gasoformans. Two strains from heart blood of frogs appeared to be related to Achromobacter multistriatum, and were non-pathogenic for frogs. One unidentified strain, motile by a single polar flagellum, which liquefied gelatin, and formed acid but not gas in glucose, was isolated from heart blood of salmon. This organism was non-pathogenic for frogs. Five strains of organisms which were motile by polar flagella, liquefied gelatin, formed indol, and produced acid and gas in glucose, saccharose, and mannite, were obtained from muscle lesions of salmon, intestine of trout, and heart blood of goldfish. These were non-pathogenic for frogs. Five strains which resembled the preceding five except that they did not form indol - and one did not liquefy gelatin - were isolated from heart blood of frogs, skin lesion of trout, and heart blood of salmon kelt. These bacilli were pathogenic for frogs, in which they caused general blood infection. Three strains which did not liquefy gelatin, form indol, or ferment sugars, were obtained from heart blood of trout. They were highly pathogenic for frogs, again producing general infection./

infection. This group of non-pigment-producing bacilli was next in frequency of occurrence to the Pseudomonas group, and appear to be important in spoilage of fish. They are probably derived from water, and many are potential pathogens.

Coliform bacilli. Four strains of atypical coliform organisms were isolated from heart blood of salmon and trout, gills of trout, and furunculous lesion of salmon. The strain from lesion of salmon was pathogenic for frogs, in which it produced a general infection. The others were non-pathogenic.

Gram-positive, non-sporing bacilli. Three strains of such organisms were isolated from heart blood and skin lesion of salmon kelt, and gills of trout. These were non-motile, and could not be identified with any known type. One motile organism, slightly larger than these, was obtained from gills of trout. These organisms were non-pathogenic for frogs, and appeared to have been derived from water.

Gram-positive sporing bacillus. One organism of the genus Bacillus was found in the intestine of a trout. It was non-pathogenic for frogs.

Vibrio. One vibrio was isolated from heart blood of salmon, and proved pathogenic for trout but not for frogs.

Yeasts. Two strains of yeast-like organisms were isolated, from intestine of trout and skin lesion of salmon. The former produced pink pigment, and a similar strain was known to be present in the water from which the fish came.

CHARACTERISATION, OCCURRENCE, AND IDENTIFICATION OF MICRO-ORGANISMS ISOLATED FROM COLD-BLOODED ANIMALS.

Note: In the succeeding descriptions of organisms, the nomenclature adopted by the Society of American Bacteriologists, as given in Bergey's Manual of Determinative Bacteriology, has been adhered to as far as possible.

Ref. No. Ss31.

Source : Isolated from skin lesion on head of salmon, summer 1927.

Morphology : Coccus, large, single, pairs, and irregular groups.

Staining reactions : Gram-positive.

Cultural characters : Aerobe. Opt.temp. - grows well at 20°C. and 37°C.

Agar slope culture. White, glistening, opaque, raised.

Agar colonies - 0·5 - 0·75 mm. in three days at 20°C. circular, dense, white, glistening, edge entire and thinner.

Gelatin stab culture. No liquefaction. Heavy surface growth, filiform in track.

Bouillon. Uniform turbidity, depositing on sides of tube, slight pellicle.

Potato. Rather scanty white growth.

Litmus milk. Slightly acid, no coagulation.

Biochemical reactions. Indol not produced.

Glucose ⊥, Lactose ?, Saccharose ⊥*, Dulcite –, Mannite ⊥*.

* very slight.

Pathogenicity : Non-pathogenic for frogs, by subcutaneous inoculation.

Identity :/

Identity : The general characters of this organism agree with those of the genus Micro-coccus (Cohn), and except for poorer growth on Potato, with those of the species Micro-coccus candicans (Flügge). The organism appears to be a strain of Micro-coccus candicans.

A similar strain (Ss4.1) was isolated from a similar lesion in another sal-:mon received at the same time.

Ref. No. T.C. d280.I.2.

Source : Intestine of trout, which died accidently.

Morphology : Coccus, large, single, pairs, and irregular groups.

Staining reactions : Gram-positive.

Cultural Characters : Aerobe. Grows at 20°C. and 37°C.

Agar slope culture. Dead white, thick, moist, smooth.
Colonies on agar - 0·5 mm. in three days at 20°C. White, raised, circular, very dense, finely granular, brownish under low power.

Gelatin stab culture. No liquefaction. Surface growth, and track finely beaded.

Bouillon. Uniform turbidity and whitish deposit.

Potato. Flat, moist, glistening, creamy.

Litmus milk. Acid and loose clot very late. (14 - 20 days).

Biochemical reactions : Indol is not produced.

Glucose $\perp^{*}$, Lactose $\perp^{*}$, Saccharose $\perp^{*}$, Dulcite –, Mannite $\perp^{*}$.

*very slight.

Pathogenicity : Non-pathogenic for frogs, by subcutaneous injection.

Identity : This organism agrees in general characters with the genus Micro-coccus (Cohn), and resembles the species Micro-coccus candicans (Flügge). It differs from that organism, as described by Flügge, in producing clot in milk, and in not forming a pellicle on bouillon.

Ref. No. T.C. d4N. G3.

Source. : Isolated from gills of trout, which died in aquarium.

Morphology : Cocci, large, single, pairs, fours and clumps.

Staining reactions : Gram-positive.

Cultural characters : Aerobe. Opt. temp. about 20°C. No growth at 37°C.

Agar slope culture. Pale yellow, smooth, moist, glistening, becoming very bright yellow.

Colonies on agar - Small, raised, dense, opaque, creamy yellow. Granular, circular, edge almost entire.

Gelatin stab culture. Yellowish growth surface and track, no liquefaction.

Bouillon. Slight uniform turbidity, yellowish deposit.

Potato. Bright yellow, moist, glistening.

Litmus milk. Slightly alkaline, dirty deposit and scum.

Biochemical reactions : Indol is not produced.

Glucose, lactose, saccharose, dulcite, mannite are not fermented.

Pathogenicity : Non-pathogenic for frogs by subcut-:aneous inoculation.

Identity : This organism belongs to the genus Micro-coccus (Cohn), and is a strain of the species Micro-coccus luteus (Schröter). It differs from the type strain by its action on Litmus milk, which is usually slightly acid.

Ref. No. 7.

Source : Isolated from muscle of furunculous salmon, August, 1926.

Morphology : Coccus, small, single, pairs and irregu-:lar groups.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. Slight growth at 37°C.

Agar slope culture. Slightly granular, whitish, moist.

Colonies on agar - 1 mm. in three days at 20°C. Circular, slightly raised, finely granular, yellowish, edge thinner and fringed in appear-:ance.

Gelatin stab culture. No liquefaction, "nail" growth.

Bouillon. Uniform turbidity, deposit, flocculi formed later.

Potato. Flat, slightly granular, pale lemon growth.

Litmus milk. Slightly reddish at surface, white deposit.

Biochemical reactions : Indol is not formed.

Practically no fermentation reactions.

Pathogenicity : Non-pathogenic for frogs, by subcut-:aneous injection.

Identity : This organism conforms to the genus Micro-coccus (Cohn), but differs from any described species.

Ref. No. 5

Source : Isolated from muscle of furunculous salmon, August, 1926.

Morphology : Bacillus, short, single and pairs, motile by peritrichous flagella - non-sporing.

Staining reactions : Slight tendency to Gram-positive staining.

Cultural characters : Aerobe. Grows at 20°C. and 37°C.

Agar slope culture. Rather scanty, flat, smooth, yellowish-orange.
Colonies on agar - Small, slightly irregularly circular, yellowish, finely granular, centre thicker and darker, edge crenate.

Gelatin stab culture. No liquefaction, yellowish surface growth and slight in track.

Bouillon. Slight uniform turbidity and yellowish sediment.

Potato. No growth.

Litmus milk. Reddish scum and sediment, peptonisation.

Biochemical reactions : Indol is not formed.

Practically no fermentation reactions.

Pathogenicity : Non-pathogenic for frogs, by subcutaneous injection.

Identity : This organism corresponds in general characters with the Flavobacterium group (Bergey et al.), but differs from any described species.

Ref. No. 13.

Source : Isolated from liver of Salmon dead from unknown cause, September, 1926.

Morphology : Bacillus, very short and thick, motile with peritrichous flagella. Non-sporing.

Staining reactions : Gram-positive.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Pale orange, glistening, opaque, thickish.

Colonies on agar - Small, creamy yell-:ow, opaque, granular, circular.

Gelatin stab culture. Crateriform to stratiform liquefaction, with thick pale orange layer on the unliquefied medium.

Bouillon. Uniform turbidity and yellowish sedi-:ment.

Litmus milk. Alkaline and peptonised.

Potato. Orange, glistening, smooth, the medium yellowish.

Biochemical reactions : Indol not formed.

Glucose ⊥, Lactose –, Saccharose ⊥, Dulcite –, Mannite ⊥.

Pathogenicity : Non-pathogenic for frogs.

Identity : The organism agrees in general char-:acters with the genus Flavobacter-:ium (Bergey et al.), but is Gram-positive. In this and other spec-:ific characters, except the colour, it corresponds to Flavobacterium sulfureum, (Bacterium punctans sul-:fureum, Zettnow).

Ref. No. T.C. d280 H.B.2.

Source : Isolated from heart blood of trout, which died in aquarium.

Morphology : Bacillus, long and thin, showing fila-:mentous forms, often slightly curved, and chains. Non-motile. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C. Grows very luxuriantly on culture media, but rapidly loses viability.

Agar slope culture. Brownish-yellow, semi-trans-:parent, moist, very vis-:cid, has both colour and consistency of syrup.

Colonies on agar - 1 - 1·5 mm. in three days at 20°C. Flat, moist, brownish, clear; under low power, very irregular, centre mottled, edge crenate and contoured.

Gelatin stab culture. Slow crateriform lique-:faction, outgrowths into medium beyond area of liquefaction, yellow sediment.

Bouillon. Slight uniform turbidity, yellowish deposit.

Potato. Profuse, spreading, flat, moist, glistening, very brilliant, clear orange-brown.

Litmus milk. Alkaline and peptonised, dirty scum.

Biochemical reactions : Indol is not produced.

(Organism died out before fermentations could be tested).

Pathogenicity : Non-pathogenic for frogs, by intra-:peritoneal inoculation.

Identity : The general characters of this organism correspond to those of the genus Flavobacterium (Bergey et al.), and it is probably related to Flavobacter-:ium arborescens (Frankland), from which it differs mainly by action on litmus milk.

Strains/

Strains of the same organism were also isol-
:ated from heart blood of another trout
(T.C. d1N. H.B.2) which died under the same
conditions, and from the stomach of a frog
(F.d.14 M/st.). These other strains also
died out rapidly on culture media, after
showing a very profuse initial growth.

Ref. No. K. 9D. H.B.4

Source : Heart blood of salmon kelt, December, 1927.

Morphology : Bacillus, very short, thick, single and occasionally in pairs, motile by a few peritrichous flagella. Non-sporing.

Staining reactions : Gram-negative, rather poorly staining.

Cultural characters : Aerobe. Opt. temp. about 20°C.

Agar slope culture. Whitish, becoming pink, smooth, moist, glistening.

Colonies on agar. - 1 - 1·5 mm. in three days at 20°C., flat, granular, edge irregular, whitish, becoming red.

Sabouraud's medium, slope culture. Pink, moist, granular, becoming bright cherry-red, with metallic lustre.

Gelatin stab culture. Funnel of liquefaction, pinkish deposit and scum.

Bouillon. Uniform turbidity, dirty deposit.

Potato. Rough, pink, becoming vivid cherry-red, nodular, glistening.

Litmus milk. Slight acid and soft clot, dirty scum.

Biochemical reactions : Indol is not produced.

Glucose +, Lactose −, Saccharose +, Dulcite −, Mannite +.

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous inoculation.

Identity : This organism belongs to the genus Serratia (Bizio). It appears to be an a-typical strain of Serratia marescens (Flügge), from which it differs by weaker pigment production on ordinary agar, and production of gas in Sacchar-:ose. There is also no odour of trimethylamine. In these differences from the type strain, it rather re-:sembles/

resembles Serratia indicus (Koch), but the source being considered, it seems more like-:ly to be, as stated, an a-typical strain of the commoner form.

Ref. No. T.C. d1N. H.B.4.

Source : Heart blood of trout, which died of accidental infection.

Morphology : Bacillus, medium size, single and pairs. Motile by peritrichous flagella.

Staining reactions: Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Whitish, slightly granular, pellicle in condensation fluid, becomes violet at foot of slope in ten days, and in one month growth and medium become brown-:ish.

Agar colonies - 0·5 - 1 mm. in three days, roughly circular, glistening, whitish; under low power, irregularly circular, granular, slightly yellowish, denser in centre, edge irregularly crenate.

Sabouraud's medium. Violet, becoming purple and almost black. Shiny.

Gelatin stab culture. No liquefaction, whitish growth surface and track.

Bouillon. Slight floccular turbidity, fragile greyish pellicle, slight greyish deposit.

Potato. Yellowish, moist, glistening, becom-:ing purple, then almost black, while medium is darkened. Growth is shiny like wet paint.

Litmus milk. Alkaline and peptonised, rapidly.

Biochemical reactions : Indol is not produced.

Glucose, Lactose, Saccharose, Dulcite and Mannite, alkaline.

Additional characters : Lœffler's serum is slowly liquefied, growth being first yellowish and then purple, and spreading in arborescent outgrowths to the sides of track.

Pathogenicity :/

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous inoculation.

Identity : This organism belongs to the genus Chromobacterium (Bergonzoni), but differs from the described species by not liquefying gelatin. In that character and by less pigment product-:ion on agar, it differs from Chromo-:bacterium violaceus (Bergonzoni), but otherwise is so similar to that organ-:ism, that it appears to be a-typical strain, and the name Chromobacterium violaceus non-liquefaciens is suggest-:ed for it.

Another strain of this organism was iso-:lated from a skin lesion on a salmon kelt. (K.9D. Sk.les.4.)

Ref. No. 2.

Source : Isolated from heart blood of furuncul-:ous salmon, August, 1926.

Morphology : Small bacillus, single and pairs, motile by 1 - 4 polar flagella. Non-sporing.

Staining reactions : Gram-negative. In the tissues often shows bipolar staining.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Yellowish, flat, moist, glistening, becoming brownish. The medium becomes green owing to diffusible pigment and later brown.

Colonies on agar. - 1-2 mm. in three days, flat, yellowish, rather irregular, finely granular.

Gelatin stab culture. Rather slow stratiform liquefaction. Medium becomes greenish and fluorescent.

Bouillon. Uniformly turbid, green, white finely wrinkled pellicle, which becomes brownish, and the medium becomes yellowish.

Potato. Thick, spreading, moist, light brown, medium darkened.

Litmus milk. Alkaline, peptonised, greenish pellicle.

Biochemical reactions : Indol is not produced.

Glucose ⊥, Lactose –, Saccharose –, Dulcite –, Mannite –.

Nitrates reduced to nitrites and gas, which appears to be Nitrogen.

Pathogenicity : Highly pathogenic for frogs, in which, by subcutaneous inoculation, it causes a general blood infect-:ion.

Identity : /

Identity : This organism belongs to the genus Pseudomonas (Migula) and to the species Pseudomonas fluorescens (Migula). It differs from the type strain by poss-:essing more than one flagellum, and by the fact that the gas produced from nitrates is not ammonia, but appears to be nitrogen.

Other sources of strains of B. fluorescens.

Other strains of this organism, differing only in the shade of pigment produced, in degree of virulence for frogs, and rapidity of lique-:faction of gelatin, were isolated from the following sources:-

(1) Heart blood of furunculous salmon, Sept-:ember, 1926, from the Kirkcudbright Dee

(2) Heart blood of two salmon from the Grimer-:sta, Lewis, August, 1926

(3) Ulcerous skin lesions on three salmon from Banffshire, in the early summer of 1927

(4) Heart blood of furunculous salmon from the Kirkcudbright Dee, July, 1927

(5) Skin lesion (injury) on salmon from the Firth of Forth, August, 1927

(6) Ulcerous skin lesion on salmon from the Tweed, August, 1927

(7) Furunculous trout from aquarium, September, 1927

(8) Skin lesion on salmon kelt, December, 1927

(9) Heart blood of experimental animals, two trout, six goldfish, and frogs which died of mixed infection subsequent to inoculat-:ion with organisms which proved avirulent.

Ref. No. S3 H.B.2.

Source : Isolated from heart blood of salmon dead from unknown cause, September, 1926.

Morphology : Bacillus, pleomorphic, single and pairs, motile by one to several polar flag-:ella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Yellowish, flat, granular, medium slightly fluores-:cent, greenish.

Colonies on agar - 1 mm. in three days at 20°C. Irregularly circular, flat, opalescent, yellowish, finely granular, edge rough.

Gelatin stab culture. No liquefaction, medium greenish, fluorescent, growth surface and track.

Bouillon. Greenish, floccular turbidity, pell-:icle late.

Potato. No growth.

Litmus milk. Alkaline.

Biochemical reactions : Indol is not produced.

Glucose ⊥, Lactose –, Saccharose –, Dulcite –, Mannite –.

Pathogenicity : Non-pathogenic for frogs, by intra-:peritoneal injection.

Identity : This organism has the general charact-:ers of the Pseudomonas (Migula) group, and is probably related to Pseudomonas putida (Migula), from which it differs by not growing on potato, and not producing odour of trimethylamine.

Ref. No. 11

Source : Isolated from liver of salmon dead from unknown cause, September, 1926.

Morphology : Bacillus, short, single, motile by peri-:trichous flagella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Dirty whitish, moist, smooth, glistening.

Colonies on agar - Small, whitish, circular, moist, centre thicker and mottled, edge almost entire.

Gelatin stab culture. Rapid liquefaction along whole length of track and gas formation.

Bouillon. Uniform turbidity.

Potato. Whitish, flat, moist, becomes brownish

Litmus milk. Whitish deposit. No change.

Biochemical reactions : Indol not formed.

(organism died out before other tests could be made).

Identity : From the characters ascertained, this organism belongs to the genus Achromo-:bacter (Bergey et al.), and to the species Achromobacter gasoformans (Eisenberg).

Ref. No. Fd. 29J.

Source : Isolated from heart blood of frog which died as a result of inoculation with B. fluorescens.

Morphology : Bacillus, short and thick, single and pairs, showing some longer forms, motile by polar flagella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Whitish, moist, flat, very viscid.

Colonies on agar - 1mm. in three days, whitish, raised, opaque, micro-:scopically brownish-yell-:ow, finely granular, edge entire.

Gelatin stab culture. Long funnel of liquefact-:ion.

Bouillon. Turbid, heavy greyish pellicle.

Potato. Yellowish, slightly spreading, moist, glistening.

Litmus milk. Acid and clot.

Biochemical reactions : Indol is not produced.

Glucose +, Lactose –, Saccharose +, Dulcite –, Mannite +.

Pathogenicity : Non-pathogenic for frogs by intra-:peritoneal inoculation.

Identity : The general characters of this organism correspond to those of the group Achromobacter (Bergey et al.), and it appears to be closely related to Achromobacter multistriatum (Wright), from which it differs by forming a pellicle on bouillon, and by greater viscosity.

A strain (F Ken.) isolated from heart blood of a frog which died subsequently to inoculation with B. salmonicida Kennet strain, which proved a-virulent, was similar to this.

Ref. No. 3.

Source : Isolated from heart blood of furunculous salmon, August, 1926.

Morphology : Bacillus, short, thick, single and pairs, motile with one polar flagellum. Non-sporing.

Staining reactions: Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Abundant, white, soft, raised, moist, smooth, glistening.

Colonies on agar - 1·5 - 2mm. in three days at 20°C. Whitish, opaque, circular, finely granular, margin entire.

Gelatin stab culture. Slow stratiform liquefaction.

Bouillon. Floccular turbidity and whitish pellicle.

Potato. Abundant, moist, "porridge-like" growth.

Litmus milk. Litmus reduced, heavy sediment.

Biochemical reactions : Indol is not formed.

Glucose +, Lactose –, Saccharose –, Dulcite –, Mannite – .

Pathogenicity : Non-pathogenic for frogs, when inoculated into the peritoneal cavity.

Identity : This organism corresponds in general characters to the genus Achromobacter (Bergey et al.), but the specific characters do not agree with those of any described species.

Ref. No. 6.

Source : Isolated from muscle of furunculous salmon, August, 1926.

Morphology : Bacillus, medium size with longer forms, motile by polar flagella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Smooth, glistening, dirty whitish, moist.

Colonies on agar - 1·5 - 2 mm. in three days at 20°C. Circular, whitish with yellowish centre, opalescent, centre thick, finely granu-:lar, edge slightly irregular, and slight-:ly contoured.

Gelatin stab culture. Deep funnel of liquefact-:ion.

Bouillon. Fine floccular turbidity, sediment, and slight pellicle.

Potato. Dirty moist yellowish growth, somewhat scanty.

Litmus milk. Slightly acid, clot, peptonised later.

Biochemical reactions : Indol is produced.

Glucose +, Lactose —, Saccharose ± (becoming al-:kaline again), Dulcite —, Mannite + (gas re-:absorbed and becomes alkaline).

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous injection.

Identity : The general characters correspond to those of the Achromobacter group (Bergey et al.), but the sugar react-:ions are unusual. B. punctatum (Zimmermann) produces gas in glucose bouillon, but that organism also forms H_2S, and has one flagellum. The organ-:ism/

organism is probably closely related to *B. punctatum*, or *B. coadunatus* (Wright), of the *Achromobacter* group.

Ref. No. S4. M2.

Source : Muscle of furunculous salmon, September, 1926.

Morphology : Bacillus, very short and thick, single and pairs, motile with a single polar flagellum. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. Slight growth at 37°C.

Agar slope culture. Abundant, dirty whitish-yellow, moist, medium be-:comes yellowish.

Colonies on agar - Small, greyish, glistening, circular, granular, edge slightly contoured and slightly rough.

Gelatin stab culture. Funnel of liquefaction.

Bouillon. Uniform turbidity.

Potato. Yellowish, granular, slightly raised, becomes dryish.

Litmus milk. Slightly acid, clot, peptonisation and reduction of litmus later.

Lœffler's serum. No liquefaction.

Biochemical reactions : Slight indol formation in ten days.
Glucose +, Lactose –, Saccharose +, Dulcite –, Mannite +.

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous inoculation.

Identity : The general characters of this organism agree with those of the genus Achromo-:bacter (Bergey et al.), except for the more active fermentation of carbo-:hydrates. It does not resemble any described species, but is of a type which has been frequently found by the writer.

Ref. No. G.F.C.

Source : Isolated from the heart blood of gold-fish, which died without experimental interference.

Morphology : Bacillus, short and thick, showing some longer forms. Single and pairs. Motile with single polar flagellum. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. Slight growth at 37°C..

Agar slope culture. Moist, smooth, glistening, yellowish.

Colonies on agar - 2 mm. in three days at 20°C. Flat, moist, yellowish, granular, edge slightly irregular.

Gelatin stab culture. Long funnel of liquefact-:ion.

Bouillon. Turbid, white pellicle and sediment.

Potato. Scanty yellowish growth.

Litmus milk. Acid and clot, peptonised later.

Biochemical reactions : Indol is produced. There is slight nitroso-indol reaction.

Glucose +, Lactose —, Saccharose +, Dulcite —, Mannite +.

Additional characters : Rabbit blood is hæmolysed.

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous inoculation. Patho-:genic for minnows, by application to scarified skin.

Identity : This organism resembles in general char-:acters the Achromobacter (Bergey et al.) group, but members of the genus do not as a rule produce gas in sugars. The organism which is most closely resembled is B. hydrophilus fuscus (Sanarelli), but that bacillus produced acid in lactose and was pathogenic for frogs. (Bergey places Sanarelli's organism in the Proteus Group).

An/

An organism (G.F. d13D.) with the same characters was isolated from heart blood of another goldfish which died after attempted infection by feeding with B. salmonicida, which was unsuccessful. This organism and B. fluorescens were present in the blood.

Ref. No. T.C. d1N. sk.les.1.

Source : Isolated from skin lesion of trout, which had died from accidental infection.

Morphology : Bacillus, rather short and thick, single and pairs, motile with single polar flagellum. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Whitish, moist, glistening, slightly spreading, slightly wrinkled.

Colonies on agar - 0·5 - 1 mm. in three days at 20°C. Raised, opaque, creamy-white, glistening, circular; under low power, brownish, centre dark, granular, with slight radial striations towards edge which is lighter and almost entire.

Gelatin stab culture. Deep funnel of liquefaction.

Bouillon. Uniform turbidity. Slightly unpleasant odour.

Potato. Yellowish, raised, nodular, medium darkened.

Litmus milk. Soft clot, reaction unchanged, becomes slightly alkaline and peptonisation occurs.

Biochemical reactions : Indol is not produced.

Glucose +, Lactose −, Saccharose +, Dulcite −, Mannite +.

Pathogenicity : Pathogenic for frogs, by subcutaneous inoculation, producing a general infection.

Identity : This organism in its general characters resembles the Achromobacter group (Bergey et al.). The fermentation reactions, however, are more active, and the bacillus differs from any described species. Other strains almost/

almost identical with this were isolated from heart blood and skin lesion of a salmon kelt, December, 1927. The strain, (K9D HB2), from heart blood of the kelt was more highly virulent for frogs, and had a strongly ammoniacal odour on agar or bouillon media.

A strain isolated from skin lesion of a trout (T.C. d1 N. Sk.les. 3.) differed in not liquefying gelatin. This strain was also pathogenic for frogs.

Ref. No. Fd 7M.

Source : Isolated from heart blood of frog which had been fed with cultures of B. salmonicida without infection by that organism, and subsequently died.

Morphology : Bacillus, of medium size, single, some longer forms, motile by polar flagella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt.temp. 20°C. No growth at 37°C.

Agar slope culture. Dirty, moist, glistening, viscid.

Colonies on agar - About 1 mm. in three days at 20°C., whitish, translucent, adhes-:ive, moist, circular, centre yellowish and denser, slightly mottled, granular, edge rather irregular.

Gelatin stab culture. Long funnel of liquefact-:ion.

Bouillon. Uniform turbidity.

Potato. Yellowish, moist, glistening, becoming brownish, dryer, and rough.

Litmus milk. Slightly acid and soft clot; later peptonisation and reduction of litmus.

Biochemical reactions : Indol is not produced.

Glucose +, Lactose −, Saccharose +, Dulcite −, Mannite +.

Additional characters : Lœffler's serum is slowly liquefied.

Pathogenicity : Highly pathogenic for frogs by intra-:peritoneal or subcutaneous inocu-:lation.

Identity : This organism in general characters re-:sembles the group Achromobacter (Bergey et al.), except in its active fermentation reactions.
A strain (F XIII) similar to this was isolated from a frog which died of accidental infection after unsuccessful attempt to infect it with XIII.

Ref. No. T.C. d280. H.B.1.

Source : Isolated from heart blood of trout which died in aquarium.

Morphology : Bacillus, of medium size, single and pairs, actively motile by one or more polar flagella. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt.temp. 20°C. No growth at 37°C.

Agar slope culture. Whitish, moist, glistening, granular, rather adherent to medium, pellicle formed on condensation fluid.

Colonies on agar - 0·5 - 1 mm. in three days, circular, white, dense, glistening, slightly raised; under low power, brown-:ish, granular with slight radial striat-:ions, darker in centre, edge entire.

Gelatin stab culture. No liquefaction, good growth surface and track.

Bouillon. Uniform turbidity, whitish pellicle becoming heavy, whitish deposit.

Potato. Thick, raised, spreading, biscuit-coloured, dryish, rough and reticu-:late, becoming a brownish-flesh colour.

Litmus milk. Alkaline, and slight peptonisation.

Biochemical reactions : Indol is not produced.

Glucose, Lactose, Saccharose, Dulcite, and Mann-:ite become alkaline, and pellicles are formed on them.

Pathogenicity : Pathogenic for frogs, by subcutan-:eous inoculation.

Identity : The general characters of this organism correspond to those of the genus Achromobacter (Bergey et al.), but differ from those of any described species.

Strains of this organism were also isolated from the heart blood of two other/

other trout which died under similar con-
:ditions, and all strains were found to be
pathogenic for frogs.
Other strains corresponding closely to this
are - T.C. d1N. H.B.3. and T.C. d1N. H.B.1.

Ref. No. T.C. d280. Int. 1.

Source : Isolated from intestine of trout which died in aquarium.

Morphology : Bacillus, medium size, single and pairs, motile by single polar flagellum. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 20°C. No growth at 37°C.

Agar slope culture. Whitish, moist, glistening, abundant, raised, slightly granular, medium darkened.

Colonies on agar - 1·5 - 2 mm. in four days, flattish, circular, moist, glistening, whitish; under low power, brownish, granu-:lar, opaque, edge rough.

Gelatin stab culture. Funnel of liquefaction.

Bouillon: Turbid with whitish pellicle.

Potato : Moist spreading growth, slightly darker than medium, flat, becoming rough and having the appearance of bubbles of gas, brownish.

Litmus milk : Acid, loose clot, peptonisation later.

Biochemical reactions : Slight indol production.

Glucose +, Lactose —, Saccharose +, Dulcite —, Mannite +.

Pathogenicity : Non-pathogenic for frogs by sub-:cutaneous inoculation.

Identity : This organism has the general characters of the Achromobacter group (Bergey et al.), but the fermentation reactions are more active. It is similar to S4 M2. etc.

Ref. No. T.C. d4N. H.B.2.

Source : Isolated from heart blood of trout which died in aquarium.

Morphology : Bacullus, pleomorphic, single and pairs, motile by peritrichous flagella.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. 37°C. Grows at 20°C.

Agar slope culture. Dirty, moist, granular, spreading.

Colonies on agar.- Very irregular, flat, greyish, granular.

Gelatin stab culture. No liquefaction. Good surface growth.

Bouillon. Uniform turbidity, greyish deposit, no pellicle.

Potato. Flat, yellowish, rather scanty.

Litmus milk Alkaline, slight peptonisation late.

Biochemical reactions : Indol is produced. Nitroso-indol reaction positive.

Glucose + , Lactose −, Saccharose + , Dulcite −, Mannite −.

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous inoculation.

Identity : This organ is an atypical non-lactose-fermenting, coliform bacillus.

Ref. No. S3 HB 1.

Source : Isolated from heart blood of salmon dead from unknown cause, September, 1926.

Morphology : Bacillus, short and thick, single and pairs. Non-motile. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt.temp. 37°C. Grows at 20°C.

Agar slope culture. Luxuriant, white, moist, raised, smooth.

Colonies on agar - 1-1·5 mm. in two days at 20°C. - opaque, pearly, raised, circular, finely granular, edge thinner than centre.

Gelatin stab culture. No liquefaction, growth good surface and track.

Bouillon. Turbid, deposit, and white pellicle.

Potato. No growth.

Litmus milk Acid in 24 hours, clot in six days.

Biochemical reactions : Indol is produced.

Glucose ⊥, Lactose ⊥, Saccharose ⊥, Dulcite −, Mannite ⊥.

Pathogenicity : Non-pathogenic for frogs, by sub-:cutaneous injection.

Identity : This organism belongs to the B. coli anæ rogenes group.

Ref. No. S4 M1.

Source : Muscle of furunculous salmon, September, 1926.

Morphology : Bacillus, single and pairs, short and thick, showing some long forms. Tends to have square ends. Non-motile. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Opt. temp. - grows well at 20°C. and 37°C.

Agar slope culture. Luxuriant, white, moist, thick, very soft.

Colonies on agar.- 1 mm. in three days at 20°C. White, glistening, raised; under low power brownish, finely granular, slight radial striations, edge entire, circular.

Gelatin stab culture. No liquefaction. Good growth surface and track.

Bouillon. Uniform turbidty and white deposit.

Potato. Profuse, white, raised, finely wrinkled, glistening.

Litmus milk. Acid and clot.

Biochemical reactions : Indol is not produced.

Glucose ⊥, Lactose ⊥ (late), Saccharose ⊥, Dulcite −, Mannite ⊥.

Pathogenicity : Pathogenic for frogs, by subcutaneous inoculation.

Identity : This organism belongs to the B. coli anaerogenes group.

Ref. No. K 9D HB1.

Source : Isolated from heart blood of salmon kelt, December, 1927.

Morphology : Small bacillus, single, pairs and in groups at angles. Non-motile. Non-sporing. Non-branching.

Staining reactions : Gram-positive Non-acid fast (decolourised by 1 per cent. sulphuric acid).

Cultural characters : Aerobe. Opt.temp. 20°C. No growth at 37°C.

Agar slope culture. Low, transparent, slightly granular, colourless growth.

Colonies on agar - Very minute, transparent, Microscopically irregularly circular, colourless, centre granular, margin irregular and contoured.

Gelatin stab culture. No liquefaction, slight growth surface and track.

Bouillon. Uniform turbidity and slight deposit.

Potato. Scanty, white, glistening, flat, membranous.

Litmus milk. Litmus reduced.

Biochemical reactions : Indol is not produced.

Glucose ⊥, Lactose –, Saccharose ⊥, Dulcite –, Mannite ⊥.

Pathogenicity : Non-pathogenic for frogs by subcut-:aneous inoculation.

Identity : The characters of this organism do not resemble those of any group or species described by Bergey. The staining reaction suggests Kurthia (Trevisan), but the other characters are entirely different. Strains K9D sk.les.2. and T.C. d4 NG2. are similar.

Ref. No. T.C. d4N. G4.

Source : Isolated from gills of trout which died in aquarium.

Morphology : Bacillus, of medium size, single, pairs, short chains, showing long forms with constrictions, as if in the process of division. Non-sporing. Motile by peritrichous flagella.

Staining reactions : Gram-positive.

Cultural characters : Facultative. Grows well at 20°C. and 37°C.

Agar slope culture. Rather scanty, colourless growth, at first moist, becoming dryish, granular.

Colonies on agar - Very small, dew-drop, becoming denser and dryer, granular, circular.

Gelatin stab culture. No liquefaction, arborescent growth in track, outgrowths into medium, slight surface growth.

Bouillon : Very slight uniform turbidity, white deposit.

Potato. Thin skin of white growth, slightly glistening, spreading.

Litmus milk. Alkaline and white deposit - litmus reduced.

Biochemical reactions : Indol not produced. Very slight growth on peptone water.

Glucose ⊥, Lactose ⊥, Saccharose ⊥, Dulcite −, Mannite ⊥, in 3 days.

Pathogenicity : Non-pathogenic for frogs, by subcutaneous inoculation.

Identity : This organism resembles the genus Kurthia (Trevisan), but members of that group do not ferment carbohydrates. Spores were not produced, so the organism does not belong to the family Bacillaceæ (Fischer). There is some resemblance/

resemblance between this strain and an organism (K9D. HB1) isolated from heart blood and skin lesion of a salmon kelt, but the latter was smaller, motility and flagella could not be demonstrated, there were no outgrowths into the medium in gelatin stab culture, and lactose was not fermented.

Ref. No. T.C. d280. Int.4.

Source : Isolated from intestine of trout which died in aquarium.

Morphology : Bacillus. Large, but shorter than B. anthracis, tends to have square ends, single, pairs and chains, sporing; spores central or eccentric; motile by peritrichous flagella.

Staining reactions : Gram-positive.

Cultural characters : Aerobe. Opt. temp. 37°C. Grows at 20°C.

Agar slope culture. Dirty whitish, abundant, rough, spreading, adher-:ent to medium.

Colonies on agar.- About 1 mm. in three days at 20°C. - whitish, irregular, granular; under low power, extremely irregular, coiled hair appearance, ridged, edge contoured, with protruding processes.

Gelatin stab culture. Liquefaction, slow, cup-shape to stratiform.

Bouillon. Uniform turbidity, deposit, no pell-:icle.

Potato. Yellowish, dryish, becomes dirty greyish brown, dry and rather membranous.

Litmus milk. Acid and clot.

Biochemical reactions : Indol is not produced.

Glucose ⊥, Lactose −, Saccharose ⊥, Dulcite −, Mannite − .

Additional characters : Rabbit's blood is haemolysed. Loeffler's serum is liquefied.

Pathogenicity : Non-pathogenic for frogs and warm-blooded animals.

Identity: This organism belongs to the family Bacillaceae (Fischer), and to the genus Bacillus (Cohn). It has the characters of the Bacillus Anthracoides group. A strain of the same organism was also isolated from the stomach of the same

Ref. No. S4 HB2.

Source : Isolated from heart blood of furunculous salmon, September, 1926.

Morphology : Short, thick rod-shaped organism, slightly curved, single and pairs, very actively motile by single polar flagellum. Non-sporing.

Staining reactions : Gram-negative.

Cultural characters : Aerobe. Grows well at 20°C. and 37°C.

Agar slope culture. Whitish, smooth, moist, glistening, becoming yellowish.

Colonies on agar - 2 mm. in three days at 20°C. Slightly yellowish, circular, thick, structureless, opaque, edge entire.

Gelatin stab culture. Rapid funnel-shaped liquefaction.

Bouillon. Turbidity and heavy pellicle.

Potato. Whitish, becomes yellowish brown, moist, glistening, abundant, smooth, raised.

Litmus milk. Acid and clot, later peptonised and litmus reduced.

Biochemical reactions : Indol is produced strongly.

There is slight nitroso-indol reaction.

Glucose ⊥, Lactose ⊥ (late), Saccharose ⊥, Dulcite −, Mannite ⊥.

Additional characters : Rabbit blood is rapidly hæmolysed.
Loeffler's serum is liquefied.
There is distinct growth on Dieudonné's medium.
The organism is killed at 60°C. in 5 minutes.

Pathogenicity : Non-pathogenic for frogs and guinea-pigs, by intraperitoneal inoculation, and for pigeons by intramuscular/

intramuscular injection. Pathogenic for trout by application to scarified skin, and less pathogenic for minnows by the same method.

Identity : This organism has the general characters of the genus Vibrio (Müller). It corresponds closely in general biologi-:cal characters to various types of vibrios which have been isolated from water and resemble the cholera vibrio in general biological characters except for their rapid hæmolytic action.

YEASTS

Ref. No. T.C. d280. Int.3

Source : Isolated from intestine of trout which died in aquarium.

Morphology : A yeast-like organism. Buds tend to remain attached to parent by processes. Spores were not obtained.

Cultural characters : Aerobe. Opt.temp. 20°C. No growth at 37°C.

There was good growth on ordinary culture media, and especially on Sabouraud's medium.

Delicate pink pigment was formed.

Gelatin was not liquefied.

Identity : Corresponds to Saccharomyces rosaceus.

Ref. No. Ss2.

Source : Isolated from lesion on head of salmon, summer 1927.

The general characters were like those of the preceding, but no pigment was produced, and there was slow liquefaction of gelatin.

IV. CERTAIN BACTERIAL SPECIES FOUND TO BE PATHO-:GENIC FOR FISH OR FROGS IN NATURE OR BY EXPERIMENTAL INOCULATION: AND FORMS OF DISEASE MOST FREQUENTLY MET WITH IN THESE COLD-BLOODED ANIMALS.

Bacillus salmonicida has been generally accepted as the specific causative organism of "Furunculosis of the Salmonidae". This organism was found to be pathogenic for trout, frogs, gold-fish, and minnows. The condition produced was a general infection.

Bacillus fluorescens was found to be highly pathogenic for frogs, by subcutaneous or intraperi-:toneal inoculation. 1/10 of an agar slope culture produced death in 3-14 days, according to the viru-:lence of the strain used. General infection occurred, and the bacilli could be demonstrated in large numbers in the blood stream. Phagocytosis, both by polymorphs and mononuclears, was noticeable, and when inoculation was subcutaneous, these cells were present in the subcutaneous fluid. In the blood, the bacilli were often in pairs, and frequently showed bipolar or irregular staining. It was found that organisms, irrespective of species, which appeared in the blood of poikilothermic animals, tended to stain in this manner.

Certain strains of non-sporing, non-chromogenic, Gram-negative bacilli, isolated from various sources, proved/

proved pathogenic for frogs by subcutaneous inocula-:tion. General infection occurred and the organisms appeared in large numbers in the blood stream. The condition produced in frogs by infection with the organisms was indistinguishable from that caused by B. fluorescens.

A vibrio, isolated from heart blood of a salmon, proved highly pathogenic for trout, to a less degree pathogenic for minnows, and non-pathogenic for frogs. Infection of scarified skin, or intramuscular in-:jection of the vibrio in trout, led to rapidly fatal bacteraemia. The fish died in 2-3 days, and the organism could be demonstrated in the blood stream. Infection of scarified skin of a minnow caused a rapidly extending necrotic lesion to be formed, and the blood stream was finally invaded by the vibrio.

Three types of bacterial disease affecting cold-blooded animals have been described above: (1) General blood infection, without production of focal lesions; (2) "Furunculosis", general blood infect-:ion with production of necrotic lesions; (3) Ulcerative skin disease. The first appears to be the most common, and is illustrated by various epi-:zootics among fish on the Continent, such as "La Peste des Eaux douces"; so-called "Red-leg" of frogs; and infections by B. fluorescens and certain non-chromogenic bacilli seen by the writer. This form of disease is usually characterised by the appearance/

appearance of superficial haemorrhages. The con-:dition is usually acute.

The disease "Furunculosis of the Salmonidae" deviates from the typical condition of general in-:fection by the formation of localised lesions in affected salmon and trout. These lesions, however, are not constantly formed, and the disease frequently runs a course of simple and rapidly fatal bacteraemia. In experimental animals, such as frogs, this is usually the case, and when lesions are produced, they appear only at the site of inoculation.

These general infections in cold-blooded animals are characterised by the very large number of bacilli which appears in the blood of affected ani-:mals. Once the resistance of the animal has been lowered and the bacilli have gained access to the body, they multiply enormously and overrun the whole body. There is remarkable lack of reaction to in-:vading bacteria, either by the blood cells or by tissues. A slight degree of phagocytosis is usually the only attempt at resistance. In the case of B. salmonicida, another characteristic feature is the presence of that organism in dense masses in the tissues.

Ulcerative skin conditions are exemplified by "Salmon Disease", which affects practically all fresh-water fish; and various cases of ulcerous conditions which occur in salt-water fish. This form of dis-:ease/

disease is of a chronic nature and is probably due frequently to secondary infection subsequent to superficial injury. In the case of "Salmon Disease", B. salmonis pestis (Hume Patterson) was said to be the specific causative organism. It seems probable, however, that there is a group of organisms similar in characters, which are present in water and are potentially pathogenic to fish. The appearance of degenerative changes in the skin of spawned salmon, or kelts, is accompanied by a high mortality. This seems to be due to the lowering of resistance to bacterial invasion, brought about by the physiologi-:cal condition of the fish at that time. It is con-:ceivable that water organisms which are avirulent to normal healthy fish act as potential pathogens, and attack such weakened fish. Skin ulcers are frequent-:ly formed in these kelts, and the condition often closely resembles "Salmon Disease". The writer iso-:lated from a salmon kelt, a bacillus (K.9D. H.B.2) which was present in the skin lesions and heart blood, and bore a strong resemblance to B. salmonis pestis. Other organisms with similar characters were isolated from trout kept in an aquarium. These fish died of general blood infections, and strains of organisms which were pathogenic for frogs were isolated from the heart blood. The unnatural conditions under which they were kept had probably lowered their re-:sistance and laid them open to infection by potential pathogens/

pathogens from the water. Injury to the skin of fresh-water fish often leads to the formation of ulcers. Hume Patterson found that B. salmonis pestis could not infect fish when the skin was intact. The known facts lead one to suspect that certain water bacilli can, in suitable circumstances, produce disease in fish, and that B. salmonis pestis is one of these. The writer has shown that B. fluorescens, a common water organism, is a potential pathogen. Certain non-chromogenic bacilli, such as those found by Sanarelli and other continental workers, B. salmonis pestis, and certain organisms described by the writer, are very similar in general biological characters, and produce the same symptoms of general infection in cold-blooded animals.

Vibrios, similar to those found in water, have been described by David, Canestrini, and Bergmann, as producing disease in fish. The writer isolated a vibrio with similar characters, from heart blood of a salmon, and found it to be pathogenic for trout. General infection is caused by these vibrios, and the condition of animals infected with them is very similar to that produced by B. fluorescens and patho-:genic non-chromogenic bacilli.

It seems certain that many water organisms are potentially pathogenic for fish, so that when fish are injured or their resistance lowered by adverse conditions, these organisms invade their tissues and usually/

usually produce a general infection. Once the bacteria have gained an entrance, they meet with little opposition from the tissues and rapidly over-:run the body.

GENERAL SUMMARY and CONCLUSIONS.

1. The summary and conclusions referable to the study of Furunculosis are given - Page 53.

2. Bacterial infection of certain lower vertebrates may be produced by specific organisms which have been found only in association with cert-:ain conditions of disease, e.g., Bacillus salmonicida, Furunculosis among the Salmonidae.

3. Organisms naturally saprophytic may, under cert-:ain conditions, become pathogenic. These organisms comprise some types frequently found in water, such as, Bacillus fluorescens and certain Gram-negative, non-sporing, non-chromogenic bacilli.

4. A vibrio, with general biological characters similar to those of vibrios which have been described in water and in diseases of fish, was isolated from heart blood of a salmon. This organism proved highly pathogenic for trout.

5. Bacterial disease of fish and frogs usually takes the form of a general infection. Focal lesions may or may not be found. In Furun-:culosis, infection may be both focal and general.

6. Bacterial disease of these cold-blooded animals frequently appears in epizootic form, e.g., "Red-leg" of frogs; Furunculosis of the Salmonidae.
Occurrence of such epizootics requires further study.

7. Secondary infections, including ante-mortem in-:fection, are of common occurrence among fish and frogs, when the resistance of the animals has been lowered by primary infection or injury. Other adverse conditions probably also predis-:pose to such infections. This requires further investigation.

8./

8. Little or no tissue reaction against invading bacteria has been observed in frogs or fish, analogous to such reaction in mammalian animals.

9. A certain degree of phagocytic activity is dis-:played in some cases of bacterial infection of frogs and fish.

10. Sources and modes of infection of lower verte-:brates generally, require further study.

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DESCRIPTION OF PLATES.

PLATE I.

Fig. (1) Naked-eye specimen showing furunculous lesion in Salmo Salar. (× 1½)

Part of the body wall is seen. Necrosis has occurred in the dermis and underlying muscle tissue, and is extending downwards. On the right, the epidermis and scales are destroyed, and the necrotic material reaches the exterior.
(The dark substance underlying the skin is necrotic tissue).

Fig. (2) Section through necrotic tissue of furun-:culous lesion, from specimen in Fig. (1). (× 200)

This shows broken-down muscle fibres with irregular, dense masses of bacteria among them.

a = dense mass of bacteria.

PLATE II.

Fig. (1) Section of kidney of frog, experimentally infected with B. salmonicida. (× 400)

A glomerulus is seen, the capillary tuft of which has been destroyed. Bacilli are present in dense clumps, and also scattered among all debris and free blood corpuscles.

a = dense mass of bacteria.

Fig. (2) Section of kidney of frog, experimentally infected with B. salmonicida. (× 1000)

A dense mass of bacilli is seen, beside a capillary containing one red blood corp-:uscle.

a = dense mass of bacilli.
b = capillary and red blood corpuscles.
c = part of wall of tubule.

PLATE III/

PLATE III.

Fig. (1) Section through body wall of frog experimentally infected with B. salmonicida. (× 150)

Part of a subcutaneous and intramuscular lesion, near site of inoculation, is seen. The muscle-bundles are degenerating, the connective tissue is breaking down and contains masses of bacteria and free blood cells.

a = degenerating muscle bundles.
b = connective tissue with free blood corpuscles and masses of bacteria.

Fig. (2) Section through lung of frog experimentally infected with B. salmonicida. (×800)

(Note: the lung of a frog is a simple sac, with highly vascular, folded walls.)

The section shows part of the vascular wall of the lung, with many bacilli in and around blood vessels. The tissue generally shows pathological changes.

a = blood vessel with corpuscles.
b = bacilli.

PLATE IV.

Fig. (1) Film of heart blood of frog, experimentally infected with B. salmonicida. (× 1200)

Two red blood corpuscles are seen, and several bacilli, some showing bipolar staining.

Fig. (2) Smear of spleen of goldfish, experimentally infected with B. salmonicida. (× 1000)

The organ was reduced to a semi-fluid condition. Large numbers of bacilli, many in pairs, and degenerating cells are seen.

PLATE V.

Fig. (1) Film of heart blood of frog, experimentally infected with "K9D H.B.2". (× 1600)

(Note/

(Note: films of heart blood of frogs experimentally infected with other Gram-negative, non-sporing, non-chromogenic bacilli, described above as pathogenic for frogs, and with B. fluorescens, are similar in appearance.)

The bacilli are present in very large numbers in the blood, and many show distinct bipolar staining.

a = red blood corpuscle.
b = bacilli showing bipolar staining.

Fig. (2) B. salmonicida, drawn to scale from film of young agar slope culture. (× 1000)

The bacilli frequently occur in pairs.

(Note: The following plates (VI - XII) consist of scale-drawings of bacteria from films of young agar slope cultures. The magnification in all cases is 1000 diameters.)

PLATE VI.

Fig. (1) Micrococcus. "TC d280. I.2".

Films of "Ss31", "T.C.d4N. G3" are simi-:lar in appearance.

Micrococcus. "7" is smaller, but simi-:lar in arrangement.

Fig. (2) Bacillus of the genus Flavobacterium "13"

PLATE VII.

Fig. (1) Bacillus of the genus Flavobacterium, "T.C. d280. H.B.2". "T.C. d1N H.B.2" and "F d14 M.st". are similar.

Fig. (2) Bacillus of the genus Serratia, probably a strain of Serratium marescens "K. 9D. H.B.4".

The arrangement of flagella in the atypical Chromobacterium violaceus strains, "T.C.d1N. H.B.4" and K.9D. Sk.les.4" is similar/

similar, but the bacilli are slightly longer.

PLATE VIII.

Fig. (1) Pseudomonas fluorescens

Fig. (2) Representative of bacilli motile by single polar flagellum, and described above as belonging to the Achromobacter group. e.g., "3", Fd 29J.", "K. 9D. H.B.2". etc.

PLATE IX.

Fig. (1) Atypical coliform bacillus, "T.C. d4N. H.B.2"

Fig. (2) Non-motile bacillus of the B. coli anaerogenes group, "S3 H.B.1".

PLATE X.

Fig. (1) Organism resembling B. anthracoides, "T.C. d280. Int. 4". Film from 12-hours' agar slope culture, showing peritrichous flagella.

Fig. (2) "T.C. d280. Int. 4". Older culture, showing spore formation.

PLATE XI.

Fig. (1) Small, Gram-positive, non-motile, non-sporing bacillus, "K. 9D. H.B. 1".

Fig. (2) Gram-positive, non-sporing, motile bacillus, "T.C. d4N. G.4.

PLATE XII.

Fig. (1) Vibrio, "S4 H.B.2".

PLATE 1.

Fig. 1.

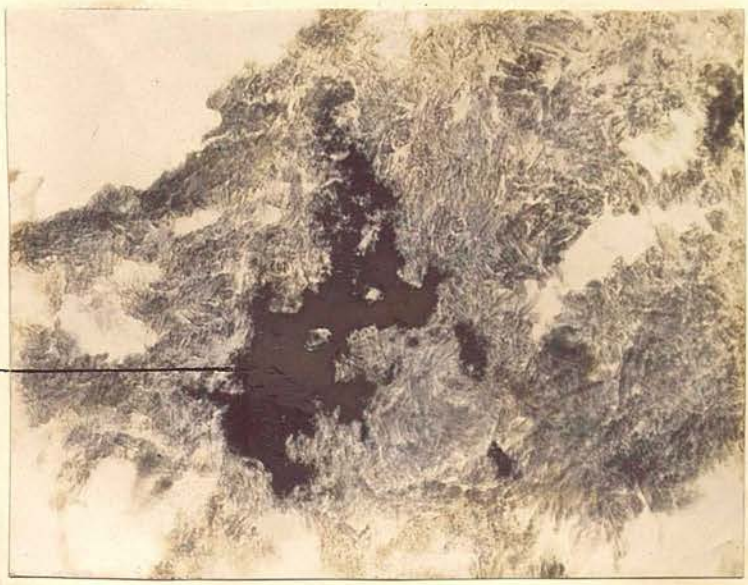

Fig. 2.

PLATE 2.

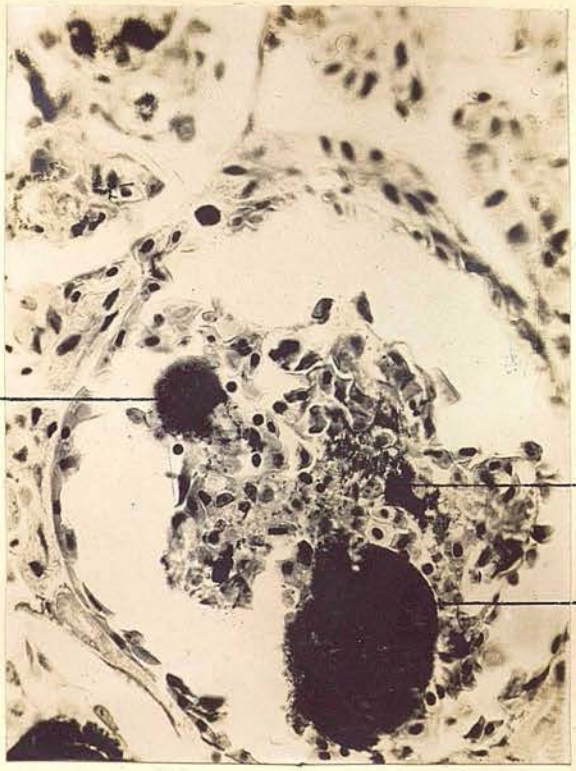

Fig. 1.

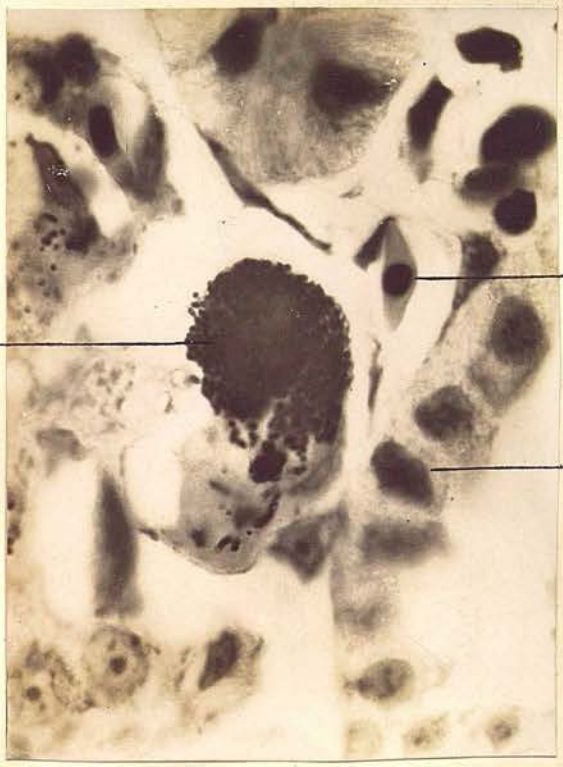

Fig. 2.

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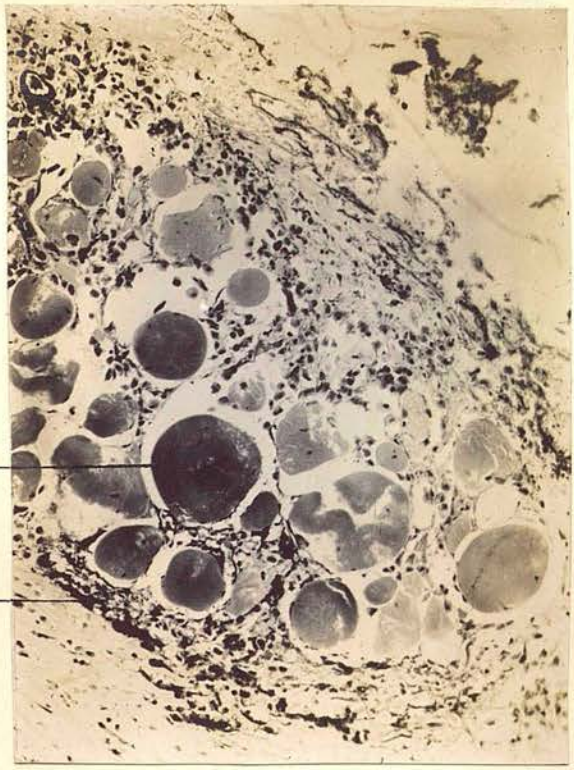

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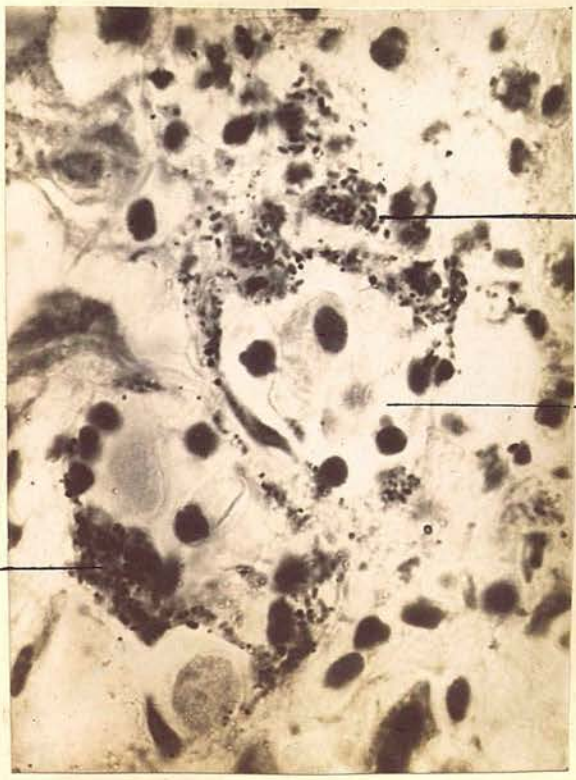

Fig. 2.

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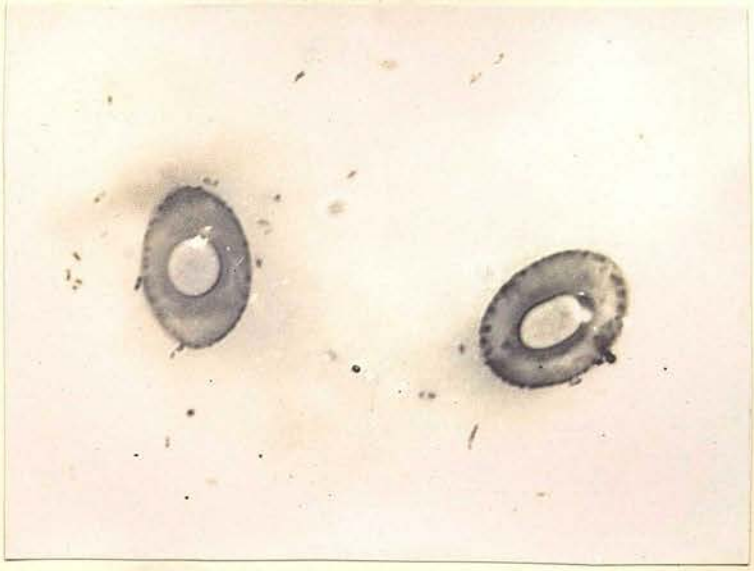

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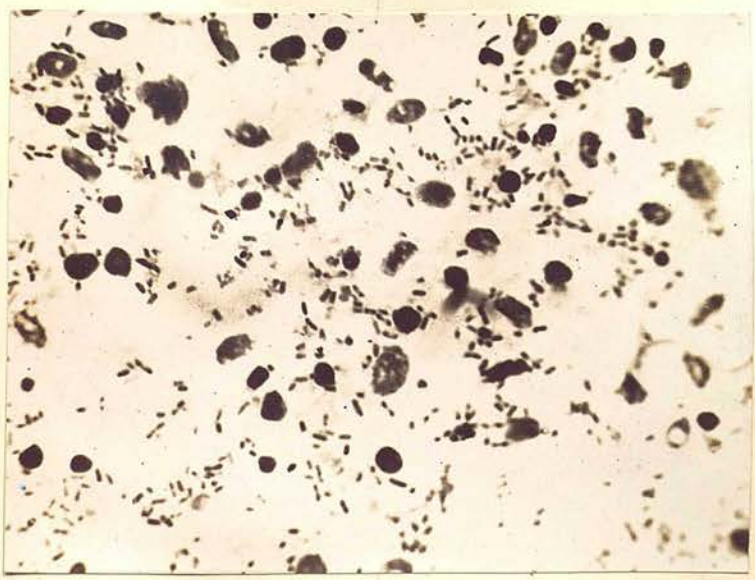

Fig. 2.

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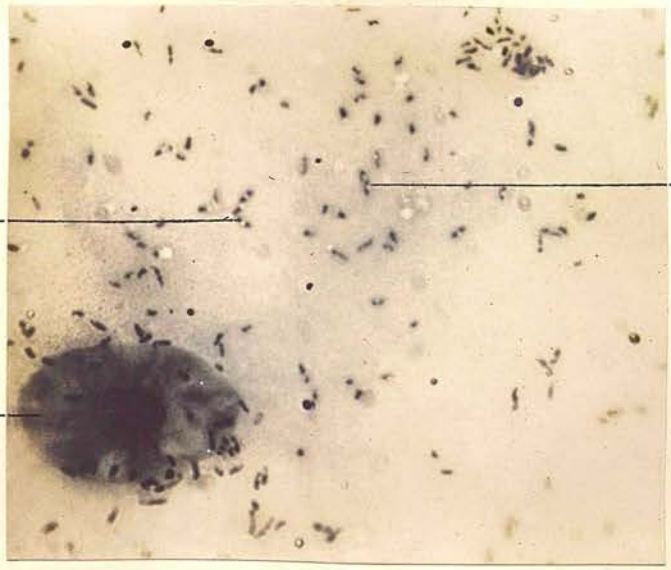

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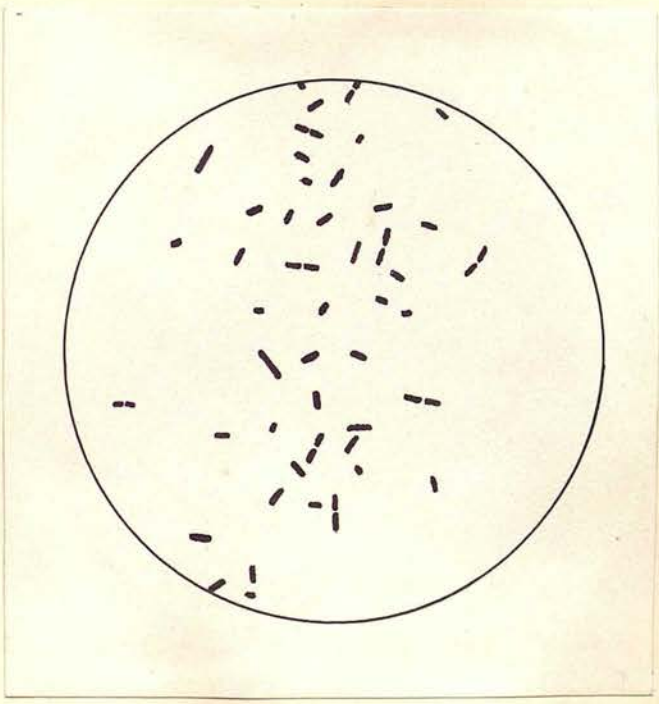

Fig. 2.

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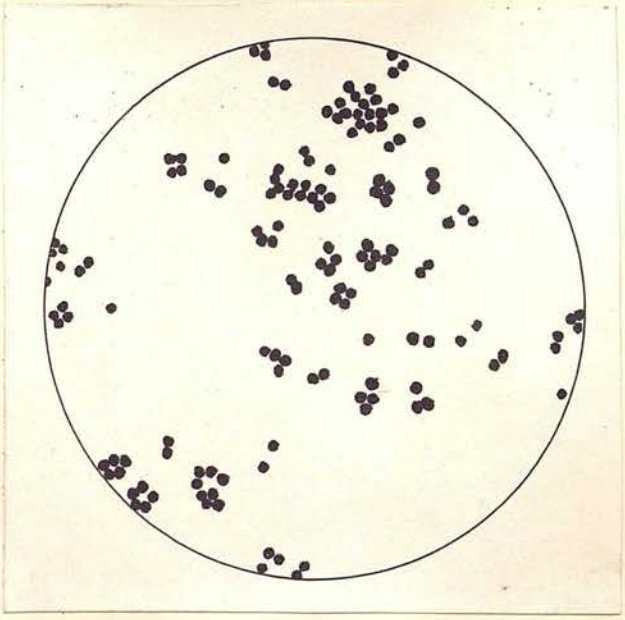

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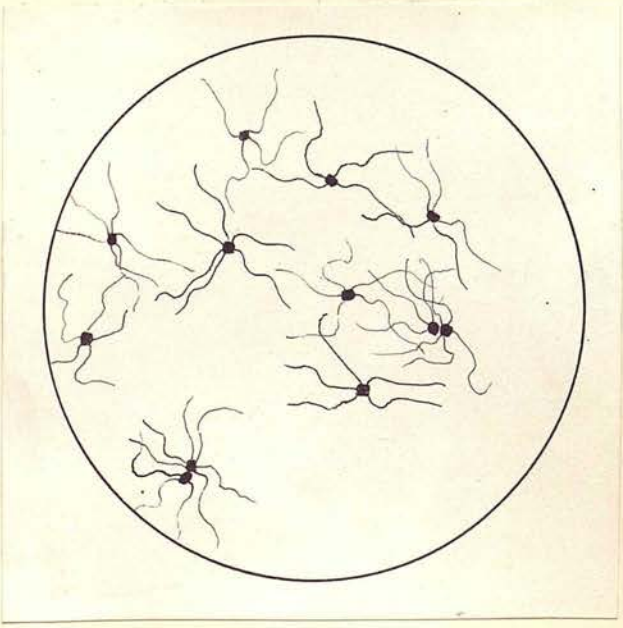

Fig. 2.

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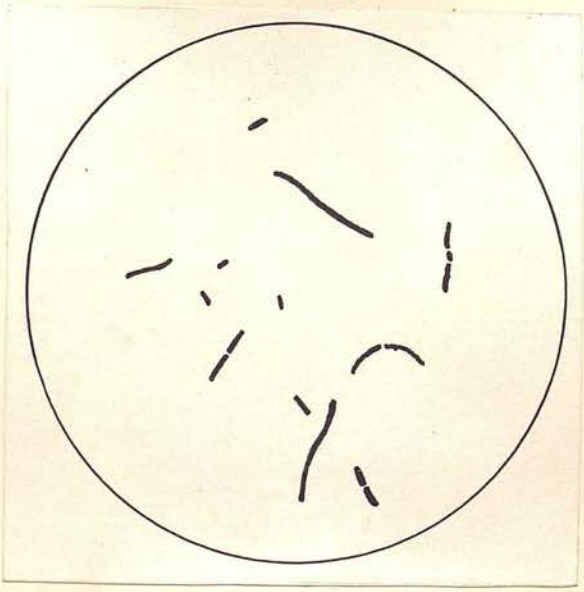

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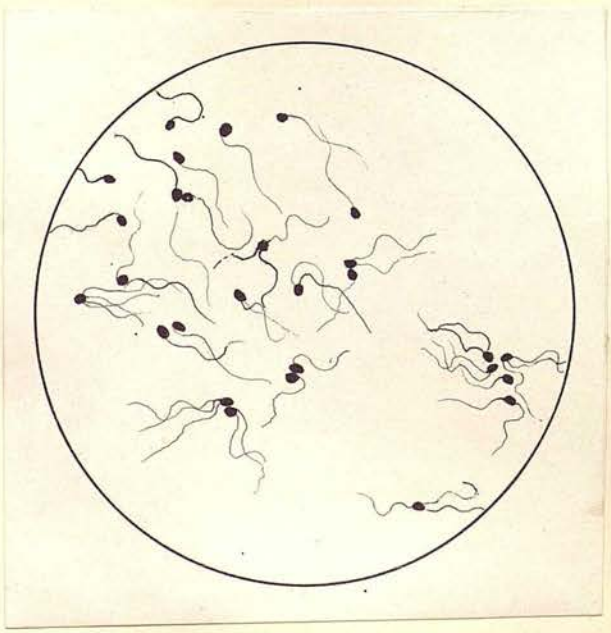
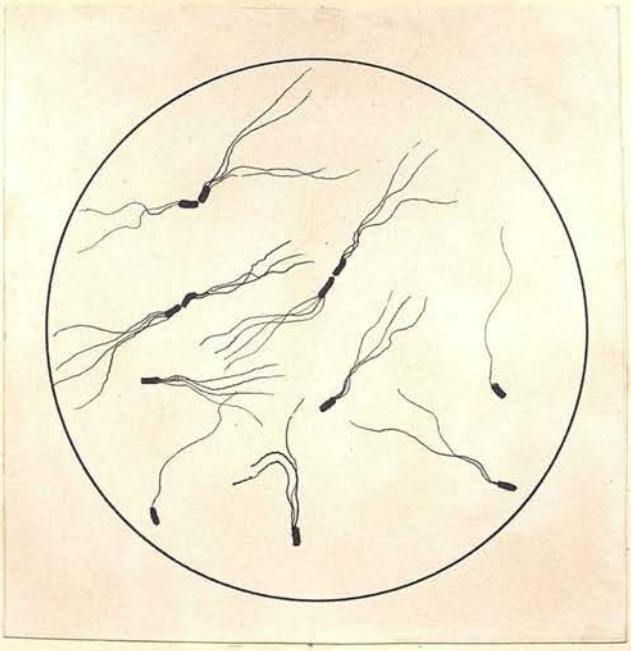


Fig. 2

PLATE 8.



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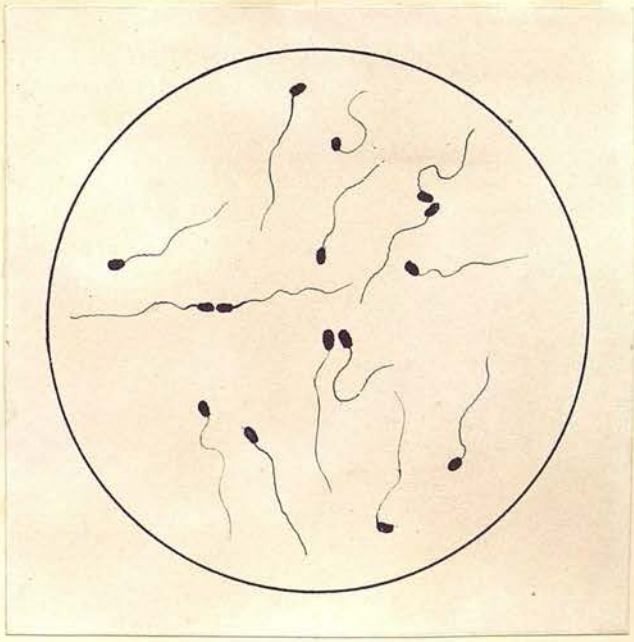

Fig. 2.

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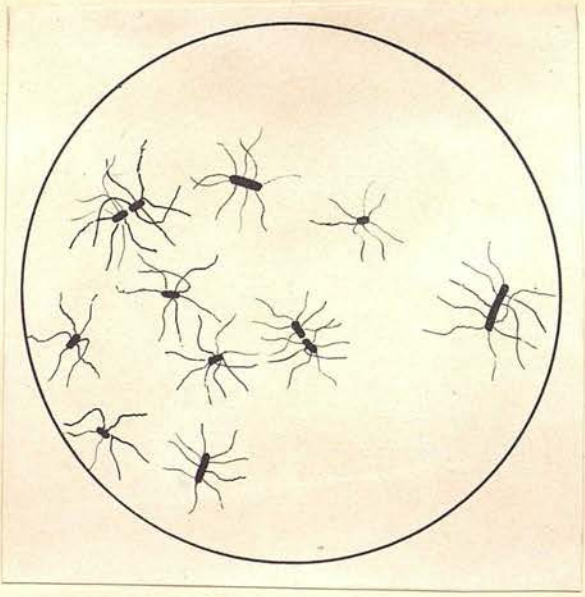

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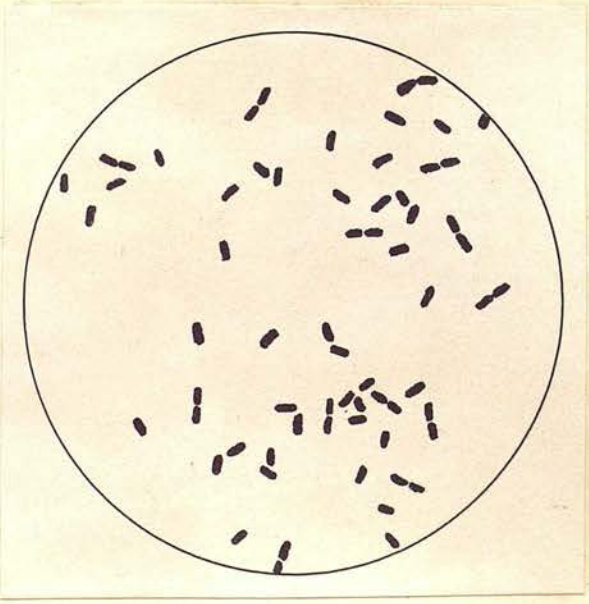

Fig. 2.

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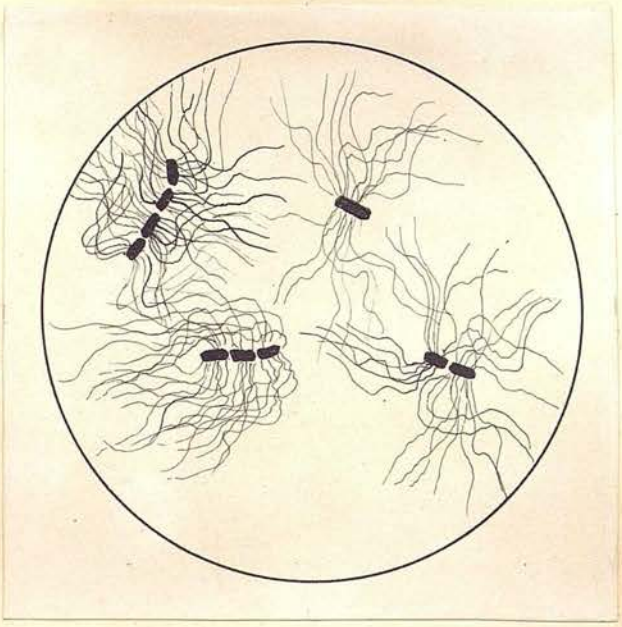

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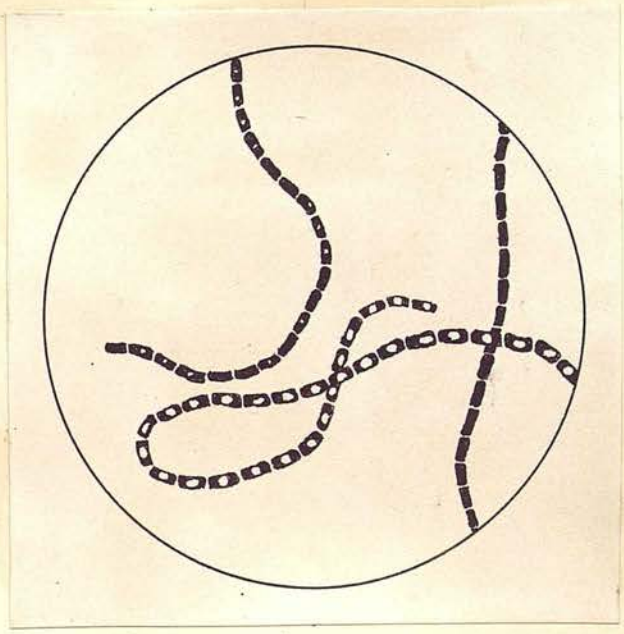

Fig. 2.

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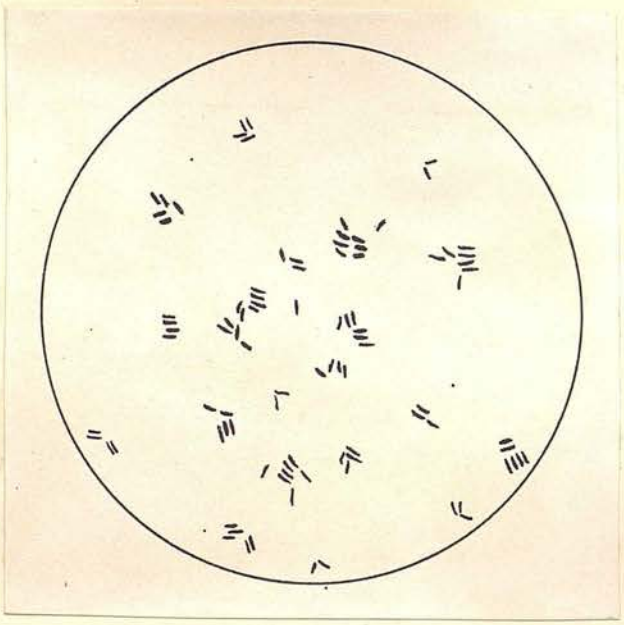

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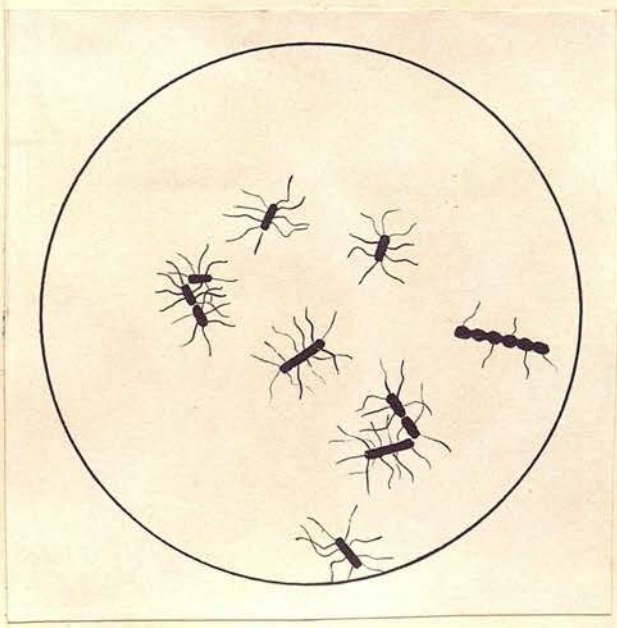

Fig. 2.

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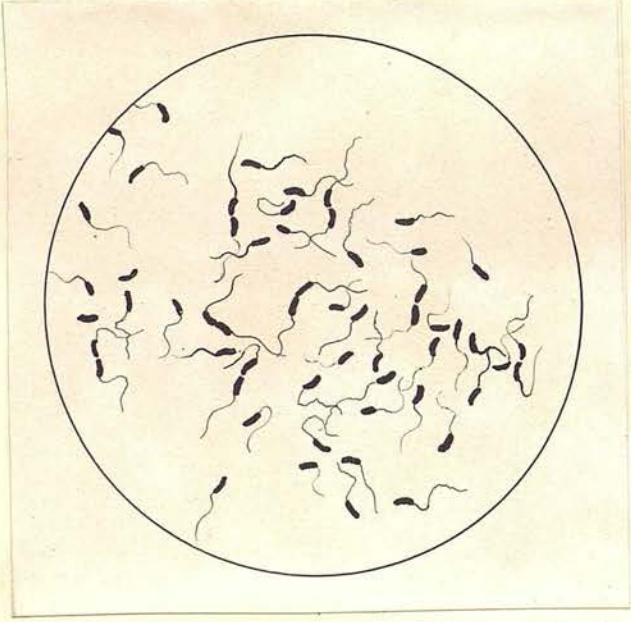

Fig. 1.