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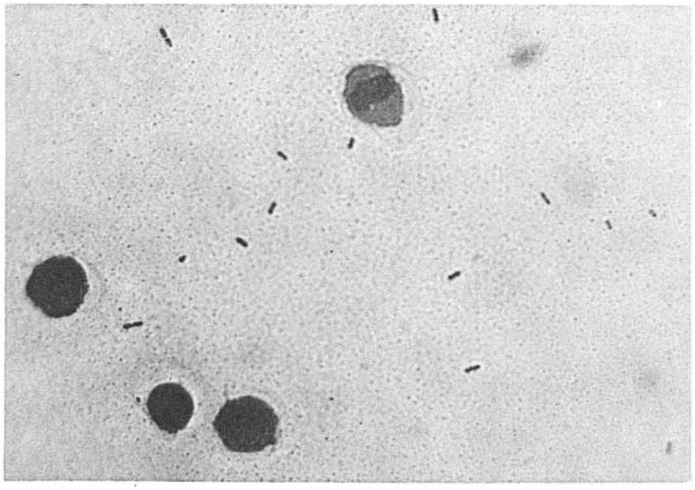

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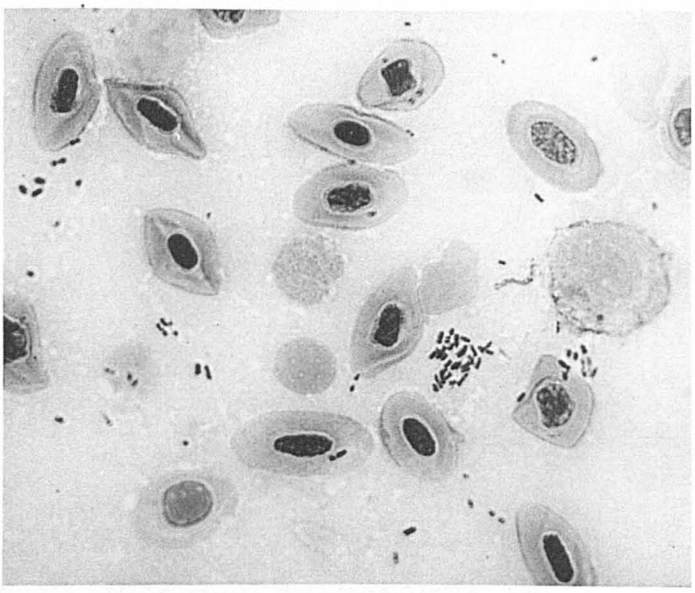

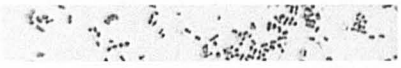

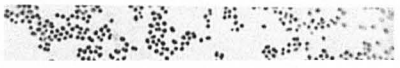

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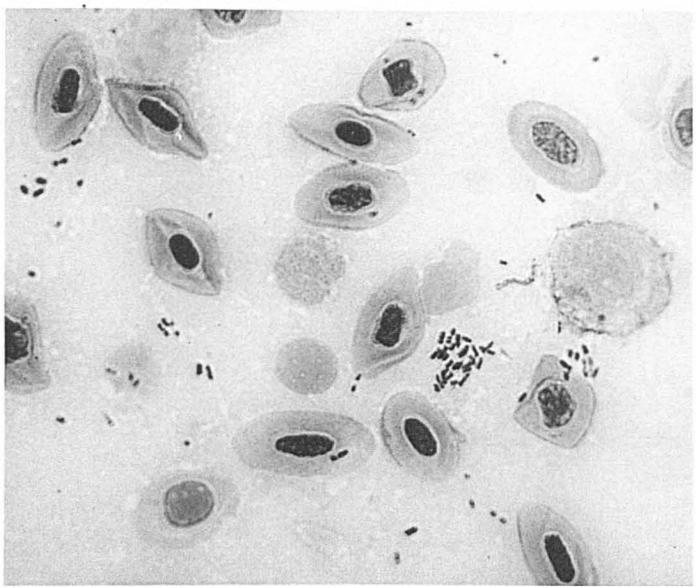


A MORE COMPLETE DESCRIPTION OF BACTERIUM TRUTTÆ

By M. C. MARSH,
Assistant, U. S. Fish Commission.

The organism here described was obtained from the blood of diseased brook trout at a station of the U. S. Fish Commission, at Northville, Mich., during the summer of 1901, and is the specific cause of the disease. The name and preliminary characterization appeared in *Science.*[a]

It is a pleomorphic form which assumes on nutrient agar-agar its simplest stage, that of a spherical or subspherical micrococcus, with occasional forms that are greater in one dimension. The strictly spherical forms are of an average diameter of 0.71μ, with extremes of 0.5 to 1.0μ, which are comparatively rare. Microscopically the field gives the impression of cocci, but bacillary individuals are frequent and reach a maximum length of 1.5μ. In liquid media, and in liquefying gelatin and blood serum, it has the form of a bacillus, and the microscopical field gives distinctly the impression of bacilli, while occasional spherical forms are intermingled. In bouillon the predominating rods are of a length from that of the diameter of a coccus up to a maximum of 2.35μ, and 0.48 to 0.83μ wide. The arrangement is frequently as diplobacilli. Many of the single rods show a slight constriction indicating their separation into cocci, while many give no sign whatever of such a structure. A few of the longer forms are slightly curved.

In the blood and local lesions of its host, the organism is in general somewhat larger than when growing on artificial media, and appears distinctly as a bacillus with occasional scattered cocci. They grow out infrequently into filaments of a maximum length of 6 μ, but the individuals average much less, and may be not longer than the diameter of a coccus, and of a width between 0.5 μ and 1.0 μ, with rounded ends. When the blood or the contents of the local lesions are plated upon agar, the resulting colonies are alike and the plate contains apparently a pure culture. All the colonies are composed chiefly of cocci, which when transferred to bouillon are transformed into a culture chiefly of apparent bacilli by the next day, or when inoculated into trout reproduce the disease and are found in the blood and lesions as bacilli. This pleomorphism is one of the most interesting characters of the species, and repeated efforts failed to reduce it to a constant form. The considerable variation in morphology in a single culture can not be removed by repeated plating, and such cultures are evidently pure, notwithstanding the variety in the form of the individuals which they contain.

[a] *Science*, N. S., Vol. XVI, No. 409, p. 706, Oct. 31, 1902.

The organism does not form spores, and a capsule has not been demonstrated. Old cultures show no marked change in the form of the organism.

Staining reactions.—It stains readily by the ordinary aniline dyes in aqueous solution. Thionin and methylene blue give excellent results. Unstained areas are occasional, but not particularly characteristic. The reaction with Gram's stain is not of much value. It stains faintly by this method, but films stained for thirty seconds in aniline-gentian-violet will retain some stain after considerable washing in alcohol, whether the iodine solution is applied or not; so that the ordinary routine of Gram's method is of little value as indicating the applicability of Gram's stain to this organism as a differential staining property. If, however, the gentian violet is applied instantaneously and then treated with the iodine solution, it is seen that the organisms retain the stain after the washing in alcohol better than control films which have not been treated with the iodine. The iodine has at least some fixing power with the gentian violet, and the organism therefore stains by Gram's method.

Cultural characters.—Growth occurs in the ordinary nutrient media, luxuriantly at a titre neutral or +0.5 to phenolphthalein; it will not grow, or but very slightly, at +1.5; at −0.5 growth is inhibited, and at −1.0 to −1.5 scarcely occurs. The following descriptions of cultures refer to media whose titre is +0.5 to phenolphthalein unless otherwise stated. The media employed were prepared from ingredients chemically pure, or as near so as the market affords. Unless otherwise stated herein the procedures[a] recommended to the American Public Health Association by its committee of bacteriologists were followed throughout, save that Liebig's extract of beef was employed instead of fresh meat.

Bouillon: A marked growth is visible after eighteen hours, without pellicle or clouding, but with the sedimenting white growth clinging to the sides of the tube. After about five days a delicate interrupted pellicle may form, and numerous flocculæ are distributed throughout the medium, both of which sink readily and upon the slightest agitation. After ten or fifteen days a characteristic brown color makes its appearance, diffusing throughout the medium, and the sediment takes on a dirty brownish color. The color deepens with age to a dark brown.

Agar-agar: On +1.5 slants it scarcely grows. After twelve days a slight multiplication is indicated by a pale filmy streak, visible best when held in the light against a dark background, and which has not increased after several weeks. The condensation water contains a slight sediment. No color is produced. Growths on agar of this titre are not sufficient to characterize the species. On +0.5 agar moderately abundant growth occurs of a grayish-white color, which with age becomes grayish brown. On usually the third day a production of a soluble pigment becomes evident, which diffuses in the medium and does not reside in the growth itself. It is a reddish-brown shade and deepens gradually until after two or three weeks it becomes a very dark brown, and the growth itself takes on a tinge of brown. In an agar-stab culture a growth of the usual features, with nothing particularly characteristic, occurs throughout the line of puncture, and an umbilicate surface growth takes place which is nearly circular and reaches a diameter of about 5 mm. in five days. Very faint color is visible by the third day, diffusing slowly downward

[a] Procedures recommended for the Study of Bacteria, etc., Jour. Amer. Pub. Health Assn., vol. 23, 1898, 56.

to a depth of about 2 cm. in seventeen days, fading gradually into the pale agar. The surface growth takes on a brownish color.

Agar plate surface colonies are round, slightly convex, the outline well defined. Microscopically they are granular, and when more than two days old the deep colonies and the surface colonies near the center become grumose. The edge of young colonies is slightly erose, but usually becomes entire or rarely broadly undulate. Well-developed colonies are translucent and yellowish under the microscope by transmitted light. Plates of about 400 colonies have, after two days, surface colonies about 0.58 mm. in diameter; after five days 0.85 mm., and then increase but little. Plates of 200 colonies have 0.54 mm. surface colonies in one day; and plates of about 25 colonies have 0.83 mm. surface colonies in two days, and after about one week these reach 3 mm. and cease to increase. Plates crowded with colonies are tinged with the brown color on the second day.

Gelatine: In +1.5 gelatine there is probably a very slight multiplication, the line of puncture showing a slight growth like a nonliquefying organism. No visible surface growth occurs and no evident liquefaction. In +0.5 gelatine abundant growth and liquefaction take place. The latter at first is either crateriform or funnelform, but may finally become stratiform, reaching the walls of the tube and extending down horizontally. Occasionally the lower end of the stab liquefies faster than the portions above it and produces a terminal sac of liquefaction. Gelatine plate cultures liquefy rapidly.

Blood serum: Blood serum is liquefied; a streak culture on solid serum results in a visible growth in eighteen hours. On the second day evident liquefaction has occurred, a shallow groove without sharply defined edges having formed, the growth sedimented on the bottom and collected at the foot of the slant with the liquefied serum. After three or four days there is a marked brown color, and the slanted portion of the serum is rapidly liquefying. After about eleven days the growth becomes slow and the color very dark brown, much darker than in old agar cultures.

Potato: On ordinary acid potato no growth occurs. On potato boiled in a known quantity of distilled water, which is then titrated and neutralized to phenolphthalein and the potato boiled in it again, there is a very slight growth. It becomes visible on the third day and appears as a faint and scanty growth of white, which is not elevated above the surface of the potato. It does not increase after four or five days and never produces color.

Milk: It grows abundantly in milk and does not cause coagulation. The reaction is unchanged or becomes slightly acid. The milk is peptonized and becomes fairly clear in from one to two weeks, and pepton may be detected in the liquid.

Dunham's pepton solution: The growth resembles that in bouillon, but proceeds more slowly. The characteristic pigment begins to be evident after about three weeks. The cultures tested gave the nitroso-indol reaction on account of indol present in the pepton. The organism does not produce indol. In Dunham's pepton solution containing rosolic acid a deepening of the pink color after about two weeks indicates the production of alkali.

Temperature relations.—The exact optimum was not determined, but it is not far from the room temperature, or 20° C. In the refrigerator at a temperature between 3° and 6° C. no visible growth occurs, but the organism is not injured; 31° C. inhibits

somewhat the growth and the human body temperature arrests it entirely and the organism is killed by an exposure to it of seventeen hours. The thermal death point is therefore low. For bouillon cultures it lies between 42° C. and 43° C. during an exposure of ten minutes.

Viability on media.—A culture on a sealed agar slant was still alive at the end of seven months. Upon transfer, however, it grew more slowly than ordinary cultures, and the pigment did not appear until between the sixth and tenth day. On the second transfer growth and chromogenic property were restored substantially to the normal.

Relation to free oxygen.—Agar plates in vacuo, by exhaustion with a Chapman pump and absorption by pyrogallic acid and caustic potash, show after two days very small microscopic colonies, while agar slants show a slight growth, neither of which increase after several days. No color appears. This incipient growth is probably due to incomplete absorption of oxygen at the beginning of the experiment, and the organism is probably an obligate aerobe.

Fermentation tests and products of growth.—It does not ferment the carbohydrates glucose, lactose, or saccharose. Cultures in 1 per cent glucose bouillon acquire an acidity, or an increase of acidity, of 1.2 per cent to 1.6 per cent in fifteen days, due probably to acetic acid, and the characteristic brown color is not developed. Lactose and saccharose bouillon show only a slight or no development of acidity, while the pigment production takes place much as in plain bouillon. The acidity apparently breaks up or prevents the formation of the pigment.

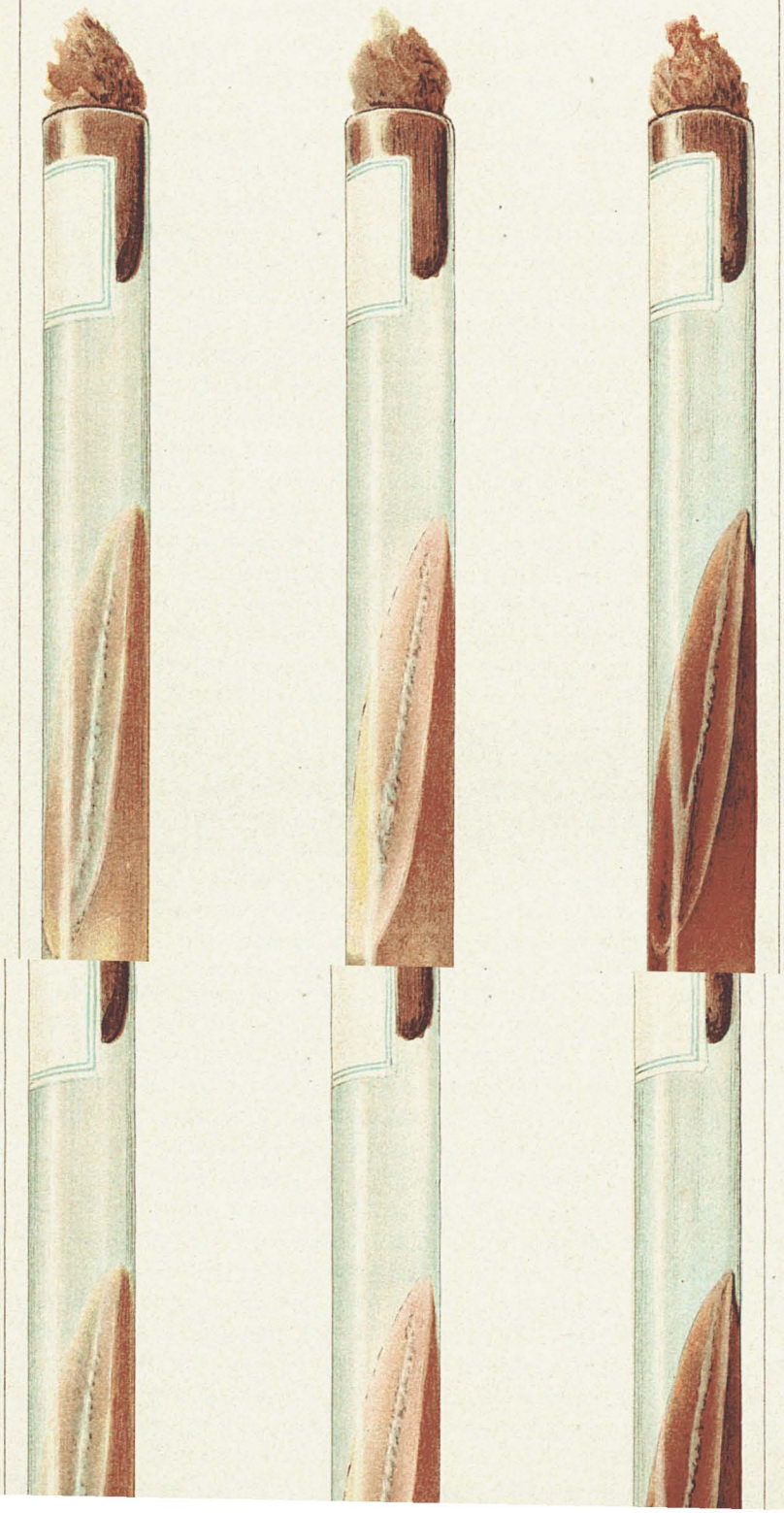
It reduces nitrates to nitrites and finally to ammonia. Seven-day cultures in nitrate broth contain both nitrites and ammonia. Forty-day cultures contain no nitrite, but give a strong test for ammonia. It does not produce indol, phenol, ammonia, invertin or diastatic ferments.

The characteristic pigment is produced in agar, bouillon, Dunham's pepton solution, and blood serum, but not in gelatin or upon potato. It is produced in alkaline, neutral, and acid media, and is inhibited by extremes of reaction, as the growth itself of the organism is inhibited. The pigment is soluble in alcohol[a] and colors the nutrient medium instead of the bacteria themselves, though with age the latter take on a shade of the color of the pigment. In liquid media and in crowded agar plates it colors uniformly the whole media, while in agar tubes the diffusion is slower, the part nearest the growth having the deepest color. It is produced at the room temperature. Higher temperatures inhibit the color faster than they do the growth. At 31½° C., which retards slightly the growth, the color is entirely inhibited, at least for a space of four days. In agar tubes the color appears on the third day, on blood serum after three or four days, in bouillon after two weeks, and in Dunham's pepton solution after three weeks.

Cultures do not have a marked odor.

Motility.—The organism direct from the blood or local lesions of the trout gives no sign of motility in the hanging drop, and its conduct in liquid media when recently isolated—a sedimenting growth without clouding—indicates nonmotility; but after it has remained for several months on artificial media and been repeatedly transferred a change takes place in its appearance in hanging drop and in its growth in bouillon.

[a] Dr. C. L. Alsberg, Harvard Medical School.



The Brownian movement is more pronounced, and a somewhat doubtful motility is suggested. Its behavior in Stoddart's medium (water, 1,000; gelatin, 5; agar, 0.5; salt, 0.5; pepton, 1) does not give a definite answer to the question of motility, the freshly isolated culture spreading scarcely beyond the point of inoculation, while cultures long in the laboratory when planted extend beyond the original inoculation, yet do not cloud thoroughly this medium. In bouillon, however, a slight but distinct clouding of the medium is observed in such cultures. A modification of habit in the line of an approach toward motility is suggested by the conduct of the organism when newly taken from its host, as compared with that when long habituated to artificial media. It is to be remembered that it circulates with the blood of the trout which it attacks, and while an active parasite in the living trout probably has little use for the power of locomotion. In artificial media the ability of the individual to change its own position would be of value. An interesting question of variation on media in the possible acquirement of motile powers is raised.

The crucial character—the presence of flagella—has not been demonstrated. Many attempts, by various methods, to stain flagella have had negative results, and for purposes of classification their absence must be assumed. For this reason and because of the morphology in the tissues of its host, which is to be regarded as its natural habitat, the organism is placed in the genus *Bacterium* as limited by Migula.

Pathogenesis.—It is pathogenic to trout, and particularly the brook trout (*Salvelinus fontinalis*), in which the disease first appeared. It has also been isolated from the Loch Leven (*Salmo trutta levenensis*) in epidemic, and in a few cases from lake trout (*Cristivomer namaycush*). It has been found thus far only in domesticated or aquarium fish and has not been seen in wild fish from the natural waters. Healthy brook trout succumb to the disease in a few days, by direct inoculation, beneath the skin, into the peritoneal cavity, or into the orbital cavity, and after a longer time by mixing cultures with their food, the organism recoverable in all cases from the heart blood. Inoculation into the dorsal lymph sac of a frog of 1 per cent of its body weight of a bouillon culture was negative, the frog showing no effects. Trout dead of the disease may be eaten, after ordinary cooking, without ill effects. A cat has habitually eaten and thriven upon the fresh, uncooked bodies of the dead trout, and the organism is probably not pathogenic for any warm-blooded animals.

Illustrations.—The colored illustration of the pigment in +0.5 agar cultures was executed by Mr. A. H. Baldwin. The photomicrographs are due to the kindness of Dr. Erwin F. Smith, who made them in the laboratory of plant pathology of the United States Department of Agriculture.