TABLE I.

	No. alive after onset							Avg	
Hours	0	1	2	3	4	5	6	Total bleeding (% of body wt)	Survival time (in hours)
1. Immobile in the supine position	7	7	7	5	0	0	0	3.9	3.1
2. Full body movement in cage									
a. Water withheld	6	6	6	6	6	0	0	4.9	4.2
b. Water *ad libitum*	6	6	6	6	6	1	0	5.0	4.5
c. Amigen and glucose by gavage	6	6	6	6	6	3	0	5.5	4.8

former. More striking were differences in the 2 groups given nothing by mouth in which there was a more pronounced drop in the hematocrit value in those immobile in the dorsal position, as compared with those allowed to move in the cage.

The most significant findings are those on the survival time and the amount bled as shown in Table I. The survival time in the controls kept immobile in the dorsal position averaged 3.1 hours. In similar experiments previously reported[2] the figure was 3.7 hours, which may have been due to a significant difference in technic, *i.e.*, ligation of the femoral artery was performed. In the present experiments the animals which were allowed to move in their cages survived from 4.2 and 4.5 hours, due to the fact that they were able to withstand at least one more hemorrhage than the controls. The beneficial effect of hydrolyzed protein and glucose by mouth is shown by the fact that two more animals survived the fifth bleeding in this group than in the next best group in which water only was ingested.

Summary. Voluntary body movement had a beneficial influence on the resistance of the dog to the fatal effects of repeated hemorrhage, as shown by a longer survival time, *i.e.*, the ability to sustain greater loss of blood, as compared with similar experiments carried out in the immobilized supine position.

2 Elman, R., and Lischer, C. E., *Ann. Surg.*, 1943, **118**, 225.

14572

Pathogenic Myxobacteria.

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During the summer of 1942 an epizootic occurred in a population of blueback-salmon fingerlings, *Oncorhynchus nerka* (Walbaum), which were being reared at the Fish and Wildlife Service hatchery at Leavenworth, Washington. The disease was recognized by Dr. F. F. Fish of the Fish and Wildlife Service as similar to, if not identical with, a bacterial disease occurring among warm-water fishes of the Mississippi Valley which was described by Davis.[1] Its occurrence as a disease of cold-water fishes has been described by Fish and Rucker.[2]

Davis gave an excellent description of the bacteria that appeared abundantly in lesions on the surfaces of infected fish, and proposed the name *Bacillus columnaris* for the species concerned. Of particular interest was his observation that when a little material was scraped from a lesion and placed in a drop of water on a slide, the bacteria present collected on the edges of infected tissues and scales to

1 Davis, H. S., *Bull. U. S. Bur. Fish.*, 1923, **38**, 261.

2 Fish, F. F., and Rucker, R. R., *Trans. Am. Fish. Soc.*, 1944, **73**, in press.

TABLE I.
Mortality of Blueback Salmon Fingerlings Exposed to Infection by Myxobacteria.

	Inoculated fish				Control
Temperature of Water in °C	16°	18°	20°	22°	22°
No. of fish	20	20	20	10	20
No. of fish dying	6	9	19	10	0
% mortality	30%	45%	95%	100%	0%

form short column-like masses. This striking movement occurred with material taken from lesions on blueback-salmon fingerlings and served to identify the infection.

Unfortunately, Davis was unsuccessful in his attempts to grow the bacteria on nutrient media. Though he obtained strong presumptive evidence of the causal relationship of *Bacillus columnaris* to the disease, failure to isolate the bacteria in pure culture rendered it impossible to demonstrate beyond question the cause of the disease. Apparently the investigation was carried no further, for no other references to this disease have been found in the literature.

The initial isolation of the bacteria, which occurred abundantly in the characteristic lesions of the disease in blueback salmon, was accomplished by serial dilution in a dilute fish-infusion medium. The bacteria failed to grow on media containing the usual concentrations of nutrients and agar but grew well at reduced concentrations. Subsequently, a medium containing 0.25% to 0.50% Bactotryptone and 0.5% to 0.9% agar adjusted to pH 7.3 was found to be very satisfactory for isolation and cultivation.

The organisms, isolated in pure culture, were recognized as myxobacteria. The vegetative cells were flexible, weakly refractive, Gram negative rods, usually measuring 0.5 to 0.7 by 4 to 8 microns. Spherical and oval microcysts varying in diameter from 0.7 to 1.2 microns were found in cultures on both liquid and solid media, and these increased in number with the age of the culture. On solid media the organisms showed the creeping motility characteristic of the myxobacteria.[3] Upon addition of a drop of water to the edge of a colony it was possible to observe the rapid flexing movements described by Bauer[4] in myxococci.

Colonies on tryptone agar appeared yellow, flat and irregular with an uneven edge in which swarming could be observed. After a few days, the colonies assumed a warty appearance with irregular elevated areas resembling immature fruiting bodies. The organisms liquefied gelatin rapidly but did not produce indol or reduce nitrates. Starch, cellulose and agar were not attacked. Sugars were not fermented, but glucose was oxidized. No growth was obtained on mineral, filtered glucose agar.

Transmission experiments under controlled conditions have shown the myxobacteria to be pathogenic to salmonid fishes. The mortality, however, depended upon the temperature at which the fish were held. Table I shows an experiment in which blueback-salmon fingerlings were placed in a dilute suspension of myxobacteria and then held in troughs. The temperature of the inflowing water was thermostatically controlled and held at the temperatures indicated. The experiment was terminated after 7 days.

It was usually possible to isolate the myxobacteria from the internal organs of infected fish as well as from lesions on the surface of the body. In one experiment typical myxobacteria were isolated in pure culture from the kidneys of 22 out of 25 fish showing the external lesions of myxobacterial infection.

A search for the source of the myxobacteria responsible for the epizootic at the Leavenworth hatchery led to the isolation of myxobacteria from well-developed surface lesions and from the internal organs of adult chinook and blueback salmon, steelhead trout, squawfish, whitefish, chubs, and suckers taken from the Columbia River. Apparently the organisms are well established in the Columbia River, and the available evidence indicates

[3] Stanier, R. Y., *J. Bact.*, 1940, **40**, 619.
[4] Bauer, E., *Arch. Protistenk.*, 1905, **5**, 92.

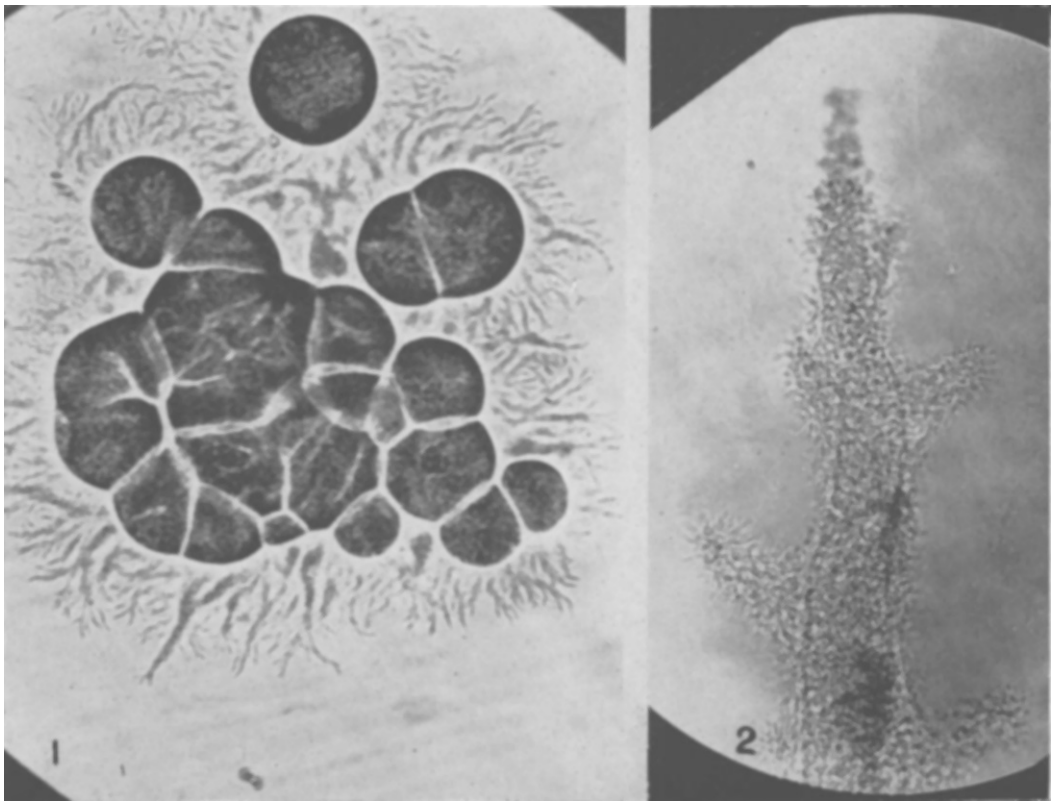

FIG. 1.
Fruiting bodies produced by *Chondrococcus columnaris* on tryptone agar. × 100.
FIG. 2.
Portion of a fruiting body produced by *Chondrococcus columnaris* in dilute tryptone broth. × 500.

that they may be responsible for a heavy mortality among native fish.

These cultures from Columbia River fishes were similar to those isolated at Leavenworth but exhibited some differences in growth on solid media. Of particular interest was the occurrence of definite fruiting bodies. These are illustrated in Fig. 1. Careful examination revealed the production of definite fruiting bodies on tryptone agar by all cultures listed above.

The phenomenon of column formation was studied using pure cultures. No column formation was observed when myxobacteria were suspended in water using ordinary wet mounts, though flexing and swinging movements were noted. In another attempt to obtain column formation, a series of cultures on sterile Brown slides was prepared. Young cultures of myxobacteria growing on tryptone agar were scraped off, placed in dilute tryptone broth on the Brown slides, and covered with sterile coverslips. In some cases sterilized scales from fish were introduced. Within 10 minutes short columns appeared at various points on the scales or at the edges of solid masses of bacteria. After 24 hours many long columns and branched structures were observed. One of these is illustrated in Fig. 2. It is apparent that these structures are fruiting bodies, since in a period of 7 to 10 days, the rod-shaped bacteria originally present were converted into spherical and oval microcysts.

The bacterial species here described clearly possesses the characteristics of the higher myxobacteria. On the basis of the shape of the microcysts the species should be assigned to the family Myxococcaceæ of the order

Myxobacteriales.[5] Following the key to the genera of the family Myxococcaceæ proposed by Stanier[5] the species should be placed in the genus *Chondrococcus,* because the fruiting bodies on agar are not deliquescent and are surrounded by a firm membrane. The species, therefore, may be properly named *Chondrococcus columnaris.* It should be pointed out, however, that the production of fruiting bodies in aqueous media is a new observation and is not considered in determining the systematic position of a myxobacterial species. It is possible that star formation, which was observed by Stapp and Bortels[6] and by Stanier[5] in liquid cultures of non-fruiting myxobacteria, is an analogous phenomenon.

We have begun an investigation of bacterial gill disease, a disease causing heavy loss of trout and salmon fingerlings. Cultures of myxobacteria have been consistently isolated from the gills of infected fish. These cultures, however, fail to produce organized fruiting bodies, and their systematic position has not yet been determined.

Summary. A myxobacterial species, *Chondrococcus columnaris,* has been isolated in pure culture and shown to produce a fatal disease in various fishes.

[5] Stanier, R. Y., *Bact. Rev.*, 1942, **6**, 143.

[6] Stapp, C., and Bortels, H., *Zent. Bakt. Parasitenk.*, 1934, II, **90**, 28.

14573

Relation of Sedimentation Rate to Amount of Precipitate Formed in Plasma by Type III Pneumococcus.*

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It has been known for many years that normal human sera contain considerable quantities of protein-polysaccharides.[1,2] According to Friedemann and Sutliff,[3] when sera from various pathologic states are inoculated with pneumococci, following an incubation period a voluminous white precipitate is obtained, while in normal sera only a slight cloudiness develops. This precipitate is not bacterial debris, but is caused by the production of unusually large quantities of acid which precipitate the serum proteins. The acids which are formed in normal sera by the organisms can be accounted for entirely by the free sugar which is fermented.[3] However, in abnormal sera growth continues at an undiminished rate after the free sugar has been completely utilized. Friedemann and Sutliff summarize their observations by stating that ". . . the phenomenon is due to the presence of abnormal quantities of a polysaccharide which supports rapid growth and which is readily fermented by the pneumococcus. It thus differs from the normally present polysaccharide, which is not apparently metabolized by the microörganism, since growth stops in normal sera when the free sugar is consumed."

These observations suggested the possibility that this abnormal polysaccharide which appeared in the blood might have some connection with the increased sedimentation rate of pathologic blood specimens. To investigate this, the following studies were carried out.

Methods and Results. Specimens of blood were obtained from a number of patients in the University Hospital, and normal blood samples were provided by members of the laboratory staff. Ten ml of blood were taken in the usual

* Submitted in partial fulfillment of the requirements for the degree of Doctor of Science in the University of Michigan.

[1] Rimington, C., *Biochem. J.*, 1929, **23**, 430.

[2] Hewitt, L. F., *Biochem. J.*, 1938, **32**, 1554.

[3] Friedemann, T. E., and Sutliff, W. D., *Science*, 1939, **90**, 335.