Aeromonads of the "hydrophila-punctata group" in freshwater fishes

by V.G. Heuschmann-Brunner

Original Title: Die aeromonaden der "hydrophila-punctata-gruppe" bei suesswasserfischen.


Translated by the Translation Bureau (VNN)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of the Environment
Fisheries and Marine Service
Biological Station
St. John's, Nfld.

40 pages typescript
### Multilingual Services Division

**Translated From** — Traduction de

German

**Into** — En

English

**Author** — Auteur

Gertrud Heuschmann-Brunner

**Title in English** — Titre anglais

Aeromonads of the "hydrophila-punctata group" in freshwater fishes

**Title in Foreign Language** (Transliterate Foreign Characters)

Die Aeromonaden der "Hydrophila-Punctata-Gruppe" bei Suesswasserfischen

**Reference in Foreign Language** (Name of Book or Publication) in Full. Transliterate Foreign Characters.

Arch. Hydrobiol.

**Reference in English** — Référence en anglais

("Archives for hydrobiology")

**Publisher** — Éditeur

not shown

**Place of Publication** — Lieu de publication

Stuttgart, Federal Republic of Germany

**Date of Publication** — Date de publication

1978

**Volume** — Volume

83

**Issue No.** — Numéro

1

**Number of Typed Pages** — Nombre de pages dactylographiées

40

**Requesting Department** — Ministère-client

DFE

**Branch or Division** — Direction ou division

Fisheries 1, Nfld. Environment Center, St. John's, Nfld.

**Person Requesting** — Demande par

D.H. Shaw

**Your Number** — Votre dossier n°

DATE DE LA DEMANDE

9 April 1979

**Date of Request** — Date de la demande

9 April 1979

**Translation Bureau No.** — Notre dossier n°

1846287

**Translator (Initials)** — Traducteur (initiales)

V.N.N.

**Date** — Date

JUN 29 1979

**Unedited Translation**

For information only

**Traduction non revue**

Information seulement
"Die Aeromonaden der "Hydrophila-Punctata-Gruppe"
bei Suesswasserfischen,"
Arch. Hydrobiol. 83(1), 99 - 125, 1978

Aeromonads of the "hydrophila-punctata group" in freshwater fishes

by

Gertrud Heuschmann-Brunner

From the Bayer. Landesanstalt fuer Wasserforschung
[Bavarian Institute for Aquatic Research]
(formerly Bayer. Biolog. Versuchsanstalt, Demoll-Hofer-Institut]
[Munich, Federal Republic of Germany]

With four Tables in the text

Summary - Aeromonads of the "hydrophila-punctata group" are ubiquitous inhabitants of surface waters. Their numbers and composition are closely related to the degree of pollution of the waters with wastes. Like certain other pseudomonads, the aeromonads belong to the natural bacterial flora of freshwater fishes. In addition, these microorganisms, however, play a significant role as potential pathogens for fishes and other poikilothermic aquatic vertebrates. Several bacteriological investigations have been performed during the last 25 years on numerous freshwater fishes with regard to their diseases, with special consideration given to the hydrophila-punctata group, since these microorganisms have gained a predominant position among the bacterial pathogens of fishes with increasing organic pollution of the waters. Since 1967, the investigations performed have been based on Schubert's taxonomy of the aeromonads, because differentiation of these microorganisms, which show little biochemical uniformity, promised better insights into the nature of Aeromonas infections of fishes. The results and factors favoring Aeromonas infections of fishes are outlined. Most strains isolated from fishes were identified as Aeromonas hydrophila ssp. hydrophila according to Bergey's Manual (1974); A. punctata ssp. punctata was represented much less frequently. After artificial infection, many of

1Dedicated to the memory of Professor Dr. Reinhard Demoll.
these strains appeared to be pathogenic for carp (Cyprinus carpio). Fish-
pathogenic strains of A. hydrophila may be found not only in diseased fishes,
but also in healthy ones, in surface water, and in sewage. Pathogenicity
of aeromonads of the "hydrophila-punctata group" depends on their ability
to produce toxins. Studies relating to this matter in fishes and other
animals as well as observations in man are reviewed.

The microorganisms of the genus Aeromonas are of particular interest
for the hydrobiologist and, specifically, for the fisheries biologist, since
these microorganisms are widely distributed partly as saprophytes and part-
ly as agents causing diseases in freshwater fishes. According to Schubert
(1967) and Bergey's Manual [of Determinative Bacteriology], Eighth Edition
(1974), three species are presently distinguished in the genus Aeromonas:
A. hydrophila, A. punctata, and A. salmonicida.

These microorganisms were discovered almost simultaneously toward the
end of the last century under different circumstances. Sanarelli (1891)
identified A. hydrophila (ssp. hydrophila) as the agent causing an aquatic
bacterial infection of frogs, and he named the microorganism isolated from
both the water and diseased frogs "Bacillus hydrophilus fuscus." In numer-
ous experiments involving the artificial infection of fishes, amphibians,
reptiles, birds, and mammals, the latter author demonstrated its pathogenicity
for both poikilothermic and homoiothermic animals\textsuperscript{1}. Diseases caused by A.
hydrophila occur in all these animal classes also under natural conditions
[in their natural habitat]. This microorganism appears occasionally even in
man as agent causing infections (Caselitz, 1966; Schubert, 1967a; inter alia).

\textsuperscript{1}The relatively low pathogenicity for birds established by Sanarelli pro-
bably is due to the higher blood temperature of birds, which amounts, on
average, to 41 to 42°C, and, thus, is located at the upper developmental
limit of A. hydrophila.
A. punctata (ssp. punctata) was found by Zimmermann (1890) in the well-
water [tapwater] of the City of Chemnitz, who named it "Bacillus punctatus." Due to its frequent occurrence in water, Kruse suggested the name "Bac. aquatilis communis" for this microorganism, which name has been accepted by several authors (cf. further below), although it never found general acceptance. A. salmonicida (ssp. salmonicida) was discovered by Emmerich and Weibel (1890; 1894) as the agent causing furunculosis in salmonids, and Lehmann and Neumann (1896) gave it the name "Bacterium salmonicida." The collective term "aeromonads of the 'hydrophila-punctata group'" covers the species A. hydrophila ssp. hydrophila and A. punctata ssp. punctata including their anaerogenous subspecies with the exception of A. hydrophila ssp. proteolytica, which, as marine and halophilous organism, cannot be expected to occur in freshwater fishes. In the present paper, "aeromonads" without more detailed denotation represent solely the members of the "hydro-
phila-punctata group," and not A. salmonicida. In the past, the names A. hydrophila (or P. hydrophila, respectively) and A. punctata (or P. punctata, respectively) have frequently been used as synonyms for the aerogenous representatives of the "hydrophila-punctata group" due to the absence of any exact definition of these microorganisms. In this connection, the European fisheries literature has been using the species name "punctata," and the American literature, the species name "hydrophila." On the basis of the results of his biochemical investigations, Schubert (1967) elaborated a taxo-
nomic system of the aeromonads, in which A. hydrophila and A. punctata are defined as separate species. We had been following the taxonomy of that author (1967; 1969) for some years before it became obligatory following inclusion in Bergey's Manual (1974), because a more detailed differentiation
of these microorganisms with respect to their role as potential pathogens for fishes appeared desirable to us (cf. page 107). One result of our investigations may be mentioned already at this time: *A. hydrophila* ssp. *hydrophila* was encountered in both water samples and fishes of different proveniences far more frequently than *A. punctata* ssp. *punctata*. The microorganisms hitherto called *A. punctata*, *P. punctata*, *B. punctatum*, Bac. *punctatus* and Bac. *aquatis communis* in the literature of fish bacteriology, thus, would be identical with *A. hydrophila* ssp. *hydrophila* to a considerable extent.

Our bacteriological investigations dealing with freshwater fishes go back to the year 1948 (when the members of the "hydrophila-punctata group" were still placed with the genus *Pseudomonas*). The following presentation deals with the aeromonads as saprophytes (pages 101 - 103) and as agents causing diseases in fishes (pages 103 ff; pp. 109 ff.). In this connection, we will refer to the distribution of the aeromonads in surface waters (pages 103 and 108). Other themes of some importance are the pathogenicity and the production of toxins (pages 118 - 120) of the different *Aeromonas* strains. The investigations carried out by us before 1967—the year of the publication of Schubert's *Taxonomy of the Aeromonads*—and those carried out after that year will be treated separately in this paper.

Peruansky (1912) was the first worker to report on the occurrence of aeromonads of the "hydrophila-punctata group" in the bacterial flora of freshwater fishes. Following investigation of the intestinal flora of fishes taken from the Neckar (River), the latter author wrote: "The aerobic bacterial species of the intestinal flora of fishes are, in part, identical with the bacteria known as inhabitants of river waters, like, for example, Bac.
fluorescens liquefaciens, Bac. aquatilis communis, ... [and], in part, they belong to the coli group. — Minkewitsch and Trofimuk (1929) investigated fishes taken from the Neva [Eiver, in Leningrad] heavily polluted with domestic waste waters (sewage), who summarized their findings as follows: "The bacillary microflora of the fish gut is composed chiefly of the groups of B. aquatilis, B. cloacae and B. coli. Apart from B. cloacae and the variants of B. coli, B. aquatilis communis must be counted with the most frequent representatives of the intestinal flora of fishes."

In investigations dealing with the Ecology of the Bacteria of the Gut of Freshwater Fishes, Mattheis (1964) was able to demonstrate a distinct dependence of the intestinal flora on both the habitat and the food of the fishes studied. Pseudomonads predominated in rainbow trout and two-year-old carp (which came mostly from pond farms), while aeromonads of the "hydrophilica-punctata group" were present in lesser numbers. In brown trout taken from a flood-water storage basin and in one-year-old carp, the aeromonads, on the other hand, occupied the first position, and the pseudomonads occupied the second position in the isolates. The other microorganisms found in the fish gut will not be dealt with in the present paper. — Trust and Sparrow (1974) investigated the bacterial flora of the digestive tract of five species of salmonids taken from 15 lakes and two smaller bodies of water in British Columbia, Canada. In 121 fishes, including 81 rainbow trout, members of the bacterial genera Enterobacter, Aeromonas and Acinetobacter were encountered most frequently. — By means of maintenance in freshwater and a certain type of nutrition, the intestinal flora of the chum salmon (Oncorhynchus keta) could be influenced so that the aeromonads predominated, with A. hydrophila occupying the top position (Trust, 1975).
Evelyn and McDermott (1961) have studied the bacterial flora of 350 freshwater fishes (mostly salmonids) taken from different Canadian waters in the Province of Ontario. Material for bacterial culture were taken chiefly from the heart, liver and kidney; more infrequently from the spleen and the muscles; and only in exceptional instances from the skin; the gut was not investigated. Demonstration of bacteria was successful in about 70 per cent of the organs. 234 of the 968 strains isolated belonged to the genus Pseudomonas, and 140 belonged to the genus Aeromonas. Enterobacteriaceae and micrococci followed next in order of frequency.

Bacteriological investigations of the liver, spleen and kidney of 998 carp taken from Dutch breeding ponds and free waters by Van der Struik (1964) yielded aeromonads of the "hydrophila-punctata group" 367 times, pseudomonads 245 times, Enterobacteriaceae 54 times, and other microorganisms in smaller numbers; 529 carp (53%) yielded positive findings.

However, the results of the investigations carried out by Evelyn and McDermott and Van der Struik, respectively, should not be generalized. Other authors (Schaeperclaus and Mann, 1939; Bullock and Snieszko, 1969) and also the present one have frequently found bacteria in the internal organs of fishes appearing healthy according to both appearance and provenience; however, the percentages of positive findings were significantly lower [than those reported by the above-mentioned workers]. In carp obtained from the Department of Fish Husbandry of our Institute, that number amounted to about 20 per cent, and was even lower in tench (Heuschmann-Brunner, 1965). The fishes were examined immediately after sacrifice, since we must expect post-mortem bacterial multiplication to occur (cf. Heuschmann-Brunner, 1970a). Chiefly aeromonads and pseudomonads were isolated from the liver, spleen and kidney.
of cyprinids; other microorganisms were rarely isolated. Since brown trout and rainbow trout had been included in that experiment for the purpose of comparison, we were struck by the finding showing that pseudomonads predominated in their organs, while aeromonads of the "hydrophila-punctata group" predominated in the [organs of] carp. The latter result probably must be attributed, first, to the non-uniform properties of the carp ponds and trout ponds in question and, secondly, to the different nutrition of these fish species. Bullock and Snieszko (1969) also found predominantly pseudomonads in the blood and kidney of 244 salmonids from two breeding farms. Material taken for culture from the kidney of brook trout, brown trout and rainbow trout yielded positive results in 12.5 per cent of the specimens of the one pond farm, and in 26.1 per cent of the other pond farm. — Our experience shows that the bacteriological findings obtained in healthy salmonids of pond farms are becoming similar to those obtained in cyprinids since the introduction of pelleted dry feeds. It is very probable that the development of aeromonads—be they in the water or in the digestive tract of the fishes—is promoted by nutrients and trace substances present in modern feeds.

Before we proceed with our outline of the relations existing between the aeromonads of the "hydrophila-punctata group" and freshwater fishes, we must first mention Schubert's investigations of the occurrence of aeromonads in surface waters (1967b) and the factors of importance for the mass outbreak of aeromonads in waste waters (sewage) (1967c), since they provide valuable conclusions regarding the ecology of this particular group of microorganisms and illustrates their saprophytic nature (cf. Heuschmann-Brunner, 1970). The aeromonads of the "hydrophila-punctata group" are typical organisms of
surface waters contaminated with organic matter. Their numbers in these waters increase with increasing pollution (contamination) with domestic waste waters. The *Aeromonas* content, thus, represents a yardstick for the waste-water contamination of a given body of water. According to Schubert (1967b) and according to determinations performed by the present author using quantitative methods of determination, the *Aeromonas* number may amount to several hundred to more than one thousand cells per milliliter water in intensively managed fish ponds, and may reach even numbers with five digits in rivers receiving untreated or inadequately purified communal waste waters (sewage). Due to their biochemical activities, the aeromonads of the "hydrophila-punctata group," no doubt, contribute to the self-purification of the waters. The aeromonads present in running waters will be dealt with in some detail further below (page 118).

Two-year-old carp kept in a pond with high *Aeromonas* content were subjected at random to bacteriological investigations over a period of several months. These investigations revealed that the aerobic intestinal flora at certain times was completely dominated by aeromonads of the "hydrophila-punctata group." The internal organs also contained occasionally *Aeromonas* cells. While alive and at autopsy, these fishes gave the impression of being overfed, but otherwise appeared healthy. No diseases were observed also in the other fishes kept in that pond.

The pathogenicity of *A. hydrophila* (ssp. *hydrophila*) for fishes—barbels and eels—has already been demonstrated by Sanarelli (1891) in the experiment. However, decades past before the aeromonads of the "hydrophila-punctata group" appeared as agents causing diseases in freshwater fishes. On the basis of his bacteriological findings and experimental investigations,
Schaeperclaus (1930; 1939; 1954) believed that *A. punctata* represented the specific agent causing a number of epidemic diseases in freshwater fishes. He attributed infectious dropsy of cyprinids (in particular, of carp), the so-called red-spot diseases of freshwater eels\(^1\) and the spot disease of pike\(^1\), perches and whitefishes to infection with that microorganism. The view held by Schaeperclaus, however, was received with some scepticism by certain fisheries research workers both in Germany and abroad, and this, in particular, because the investigations of those fish diseases in a certain institute in southern Germany as well as in laboratories of several neighboring countries yielded, in part, results differing from those reported by him. In Norway, Austria and in Switzerland, pike suffering from the spot disease exhibited not aeromonads but fluorescent pseudomonads as agents causing the infection (cf. Brunner and Reichenbach-Klinke, 1961); investigations of red spot-diseased eels carried out at the Bavarian Biological Research Establishment, in Munich, showed that a part of the animals exhibited aeromonads of the *hydrophila-punctata* group, while another part, on the other hand, exhibited (fluorescent) pseudomonads in the isolates. We obtained alternating findings also in infectious dropsy of carp, which disease we have been investigating for years (Heuschmann-Brunner, 1965). Until the early fifties, pseudomonads of the *fluorescens* group predominated in the bacterial infections of the internal organs of diseased carp from pong farms in southern Germany; aeromonads occupied the second position as agents causing diseases in the region supervised by our Institute. In the course of time, the in-

\(^1\) *Vibrio anguillarum* has been held responsible for both the red-spot disease of eels and the spot disease of pike in brackish water as well as seawater.
Infections caused by aeromonads of the "hydrophila-punctata group" have become more numerous, and finally are exceeding the infections caused by *Pseudomonas* in frequency. That change may be regarded as a consequence of the increasing pollution of the waters with waste waters. Apart from unequivocal *Aeromonas* or *Pseudomonas* infections, dropsy-diseased carp occasionally also exhibited highly mixed bacterial infections, not permitting immediate identification of the main agent causing the infection. The animal experiment helped to resolve such cases.

Experimental work aimed at determining the pathogenicity of *Aeromonas* and *Pseudomonas* strains isolated from diseased carp using both carp and tench played an important role in our investigations. For that work, we have rinsed fresh agar cultures with sodium chloride solution physiological for fish (i.e. 0.65%), adjusted the bacterial suspension to a certain translucence, and injected 1.0 to 1.5 mL of that material into the dorsal lymphatic space located under the dorsal fin of the fishes used. As a rule, a small number of additional animals were given an intraperitoneal injection of bacterial material in the same dose. Since we had to take into account the possibility of immunity to these microorganisms due to the frequent presence of aeromonads and pseudomonads in fishes, we have always used several fishes simultaneously for each test of pathogenicity. This experimental work using both carp and tench produced large differences in pathogenicity of the *Aeromonas* strains as well as of the *Pseudomonas* strains; the spectrum of pathogenicity, in fact, encompassed the range from highly pathogenic to non-pathogenic. In his material, Schaeperclaus had also encountered non-pathogenic strains of *A. punctata* in addition to strains pathogenic for fishes. Following injection of highly pathogenic aeromonads or pseudomonads, carp and tench became diseased with edemata, exophthalmus, peritonitis, and ascites,
which manifestations were usually associated with enlargement of both the spleen and the kidney as well as, occasionally, with petechial hemorrhages into the wall of the air bladder or in the dorsal muscles. The more severely diseased fishes died within a few days. The same pathological manifestations are observed also in infectious dropsy of cyprinids. However, there exists a significant difference between the disease seen after injection of bacterial material and the natural disease of infectious dropsy. In our own investigations involving the keeping of infected and healthy carp together in one aquarium, the disease caused by injection of bacterial material turned out to be non-infectious over experimental periods of several weeks. In contrast, infectious dropsy was distinguished by high contagiousness (infectivity), which, when keeping diseased and healthy fishes together, led within eight to ten days to infection of carp until then healthy, and to death of the experimental animals after about three weeks (Heuschmann-Brunner, 1965).

Our bacteriological findings as well as our animal experiments have demonstrated that the aeromonads of the "hydrophila-punctata group" may by no means be regarded as the specific pathogens of infectious dropsy of carp; representatives of the genus Pseudomonas also are not involved as causative agents. Our results rather give rise to the suggestion that infectious dropsy of carp represents primarily a viral disease with secondary bacterial infection (Brunner, 1961; Heuschmann-Brunner, 1965). On the basis of their respective findings, Russian and Yugoslav workers arrived at the same conclusion. In Yugoslavia, these researchers were Tomasec and his coworkers (1951; 1964; 1966). In 1971, Fijan et al. finally described a virus, Rhabdovirus carpio, as the specific agent causing infectious dropsy of carp. The synergism between virus and bacterial agents is as yet unclear in its details.
Investigations of this nature should include also pseudomonads of the fluorescens group in addition to aeromonads of the "hydrophila-punctata group." Pseudomonas strains, which had been isolated in the Biological Research Institute, in Munich, during an epidemic outbreak, and had turned out in the animal experiment to be pathogenic for fishes, were investigated at the Center of Hygiene, in Frankfurt a.M., at Schubert's request, using recent biochemical procedures, and determined, to a large extent, to be P. fluorescens Biovar B (personal communication).

Infections with Aeromonas or Pseudomonas also represent neither the sole nor the primary cause of the spot disease of pike and the red-spot disease of eels. Lesions of the epidermis and of the intestinal mucosa (as caused by zooparasites or other agents) may represent the sites of entry for these microorganisms, which are then readily transported into all organs by way of the blood and lymphatic circulations. Apart from lesions, infectious processes advancing from the external surface of the body, from the gills or from the gut are promoted furthermore by a lowering of the resistance of the epithelial cells. Generally speaking, and injury or lesion of the organism may lead to a lowering of its natural resistance, which is reflected, inter alia, in reduced resistance to pathogenic agents. The fish organism faces particular risks during periods of high physiological demands as, for example, during the spawning period. For that reason, certain infectious diseases—like the spot disease of pike—are seen during the spawning period or right afterwards. For the carp, originally used to a warmer climate, the winter and early spring represent a critical season in our latitudes. Outbreaks of infectious dropsy—which has been described by Fijan et al. (1971) directly as spring viremia—occur during the spring. — In pond
farming, it was customary in the past to keep carp and tench over the winter in ponds containing little food, so that these fishes were fasting more or less strongly for several months between the harvesting of stock in the fall and the harvesting in the spring. This non-biological maintenance of cyprinids led to injuries of the intestinal epithelium, consisting of local separations and defects of the mucosa, promoting bacterial infection advancing from the gut. Carp that had survived being kept like that over the winter frequently exhibited adhesions of the peritoneum, suggesting past occurrence of peritonitis. At the same time, we found in these fishes frequently colonization of the internal organs with bacteria—usually aeromonads of the "hydrophila-punctata group"—and pseudomonads. It appeared that these carp had come through a bacterial infection of the abdominal cavity with peritonitis, which was already subsiding at the time of the investigation (during April and May).

A complex of injurious factors is responsible for numerous diseases of freshwater fishes, in which aeromonads of the "hydrophila-punctata group," pseudomonads or mixed infections of the latter microorganisms occur. Following thorough analysis of the factors causing this state, one could in many cases speak of opportunistic infections.

Despite the knowledge gained in the course of our investigations of diseased and healthy fishes, it appeared to us that the bacteriological results were not really satisfactory. Differentiation of the aeromonads of the "hydrophila-punctata group," which are not uniform in their biochemical behavior, promised better insights into the nature of these bacterial infections, and would, perhaps, contribute to the elucidation of the etiology and epidemiology of certain diseases. Our subsequent investigations of
diseased and disease-suspect fishes were for that reason based on Schubert's work dealing with the biochemistry and taxonomy of aeromonads (cf. in the Bibliography).

Material taken from the organs of freshly killed fishes or fishes died naturally very recently was smeared on desxtrin-fuchsin-sulfite (DFS) medium according to Schubert (1967b) as well as on solid agar medium or trypticase soy agar medium. The cultures on DFS medium were incubated for about 24 hours at 30°C, and those on solid agar and trypticase spy agar medium, for 48 hours at 22°C. Following incubation, a relatively large number of colonies grown on these media was isolated and—after microscopic examination (staining after Gram; motility)—differentiated by biochemical means, for which purpose we have made use of Schubert's (1960; 1962; 1963; 1964; 1964a; 1969) methods of investigation. Gram-negative rods with positive cytochrome oxidase reaction and oxidative plus enzymatic hydrolysis of glucose (medium after Hugh and Leifson, aerobic and anaerobic) were tested with respect to the following metabolic activities: production of acid and gas in glucose indicator bouillon (using Durham tubes); of gas in glycerin bouillon (using Durham tubes); of arginine dehydrogenase (Møller's method); of gelatinase; of indole from tryptophane bouillon (0.1%); and of acetylmethylcarbinol (using Barrit's modification); and with respect to the reduction of nitrate to nitrite (Kauffmann's method). Most reactions were tested at 30°C; only the demonstration of arginine dehydrogenase and the detection of production of gas from glycerin were performed at 20°C. The behavior on ammonium citrate agar (Simmonds), the production of hydrogen sulfide from peptone water (2.5%), and the utilization of malonate (after Shaw and Clarke) were tested frequently, but not regularly. Initially, demonstration of butanediol dehydrogenase
had to be omitted at the start of our investigations, since the meso-2,3-
butanediol required for that purpose was at that time not yet available in
the purity desired. A part of the reactions could be performed with m-bu-
tanediol kindly made available to us by Professor Schubert\(^1\). Later on, we
have used with good success high-purity 2,3-butanediol manufactured by
Fluka AG, Buchs SG. — In special cases, i.e. in those where the eval-
uation of a disease made that step appear desirable, testing of carbohydrate
fermentation was expanded to include lactose, arabinose, cellobiose, and
sorbitol in the case of strains of \textit{A. hydrophila} ssp. hydrophila.

The aeromonads of the "\textit{hydrophila-punctata} group," as a rule, possess
a polar flagellum and are largely motile. In isolated instances, we may en-
counter microorganisms producing a brown water-soluble pigment. For instance,
Ross (1962), in the U.S.A., and Paterson (1974), in Canada, have described
pigment-producing strains isolated from diseased salmonids under the name of
"\textit{A. liquefaciens}" (ATCC 14715), which, according to our re-examination and
the recent outline of taxonomy, must be addressed as \textit{A. hydrophila} ssp. hy-
drophila biotype I (Heuschmann-Brunner and Popp, 1976). Leclerc as well as
we have found in the water non-motile strains of \textit{A. punctata} ssp. caviae
growing in brown colonies (Schubert, 1964a; Heuschmann-Brunner and Popp,
1976). The aeromonads of the "\textit{hydrophila-punctata} group" are closely inter-
related. The biochemical reactions most important for the differentiation
of their species, subspecies and biotypes are listed in Table 1. \textit{A. hydro-
phila} ssp. hydrophila (biotype I) must be regarded as the prototype of these

\(^{1}\text{Zentrum der Hygiene, Abteilung fuer Allgemeine und Umwelthygiene [Center of}
\text{Hygiene, Department of General and Environmental Hygiene], Frankfurt}
a. M., Federal Republic of Germany}
Table 1 - Differentiation of the Aeromonas species, subspecies and biotypes of the "hydrophila-punctata group." Key: 1, Aeromonads of the "hydrophila-punctata group;" 2, Butanediol dehydrogenase; 3, Gas formed from glycerin; 4, Gas formed from glucose; 5, Voges-Proskauer test (VPT).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophila subsp. hydrophila Biotyp I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. hydrophila subsp. hydrophila Biotyp II</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. hydrophila subsp. anaeogenes Biotyp I</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A. hydrophila subsp. anaeogenes Biotyp II</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. punctata subsp. punctata</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. punctata subsp. caviae</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Microorganisms; it may be imagined that the other members of this group have evolved from that prototype due to the loss of several biochemical properties. (Only A. hydrophila ssp. proteolytica occupies a special position.)

In order to obtain first an overview of the composition of the natural aeromonad flora of apparently healthy freshwater fishes, we examined—specifically for the presence of these microorganisms—smears from both the gills and the intestinal mucosa of eight carp (Cyprinus carpio), four tench (Tinca tinca), and twelve rainbow trout (Salmo gairdneri) obtained from a pond farm free of infection. From the dextrose-fuchsin-sulfite medium, we isolated 160 of the colonies grown, and subjected them to biochemical differentiation. The results revealed that 150 strains were aeromonads of the "hydrophila-punctata group," 15 strains were pseudomonads, and 17 strains were Enterobacteriaceae. Differentiation of the aeromonads yielded 112 times A. hydrophila ssp. hydrophila (82 times biotype I, and 30 times biotype II),
seven times *A. punctata* ssp. *punctata*, and nine times anaerogenous aeromonads, which partly gave a positive Voges-Proskauer test (VPT) reaction, and partly a negative VPT reaction, i.e. these aeromonads had to be assigned to different position in the taxonomic system. The natural bacterial flora of the pond fishes, thus, encompassed a wide spectrum of aeromonads of the "hydrophila-punctata group" with predominance of *A. hydrophila* ssp. *hydrophila* —a finding reminding one of the results obtained in studies of the intestinal flora of frogs during the aquatic phase of their life (Kexel and Schubert, 1967).

For our investigations of diseased fishes, we selected 14 carp (*Cyprinus carpio*), five tench (*Tinca tinca*), one grass fish [white amur] (*Ctenopharyngodon idella*), four European eels (*Anguilla anguilla*), 16 rainbow trout (*Salmo gairdneri*), three brown trout (*Salmo trutta*), and two brook trout (*Salmo fontinalis* [*Salvelinus fontinalis*; Transl.]), which reached our Institute either alive or well preserved (not frozen!); these fishes had been tentatively diagnosed as being affected by bacterial infection. Material was transferred for culture mainly from pathologically changed organs. However, since macroscopic findings may be misleading, we have always included also other organs in our investigations. In that way, we were able to determine also whether a septicemic disease was present. In most cases, we examined the liver, the spleen and the kidney, and, on an irregular basis, the cardiac blood, the air bladder, the gonads, the pancreas, the intestines, and the muscles. Material from the organs of these diseased or diseased-appearing fishes was smeared on DFS medium as well as on solid agar or on trypticase soy agar medium. The subsequent course of the examination was as described further above (page 107).
We have isolated 852 strains from these fish organs, and, where possible, differentiated these strains (cf. Table 2). Poorly growing microorganisms and microorganisms identifiable only with difficulty are not included in that number, and may be left unconsidered, since they are of no importance for the overall pattern. Infections with A. salmonicida were not found in these fishes. More than 72 per cent of these strains, viz. 616 strains, belonged to the aeromonads of the "hydrophila-punctata group." Next followed Enterobacteriaceae with 114 isolates, and pseudomonads (splitting glucose by oxidative means) with 109 isolates. Vibrios were present only in sparse numbers (13 isolates). The aerogenous aeromonads of the "hydrophila-punctata group" were differentiated as follows: 535 A. hydrophila ssp. hydrophila strains (399 biotype I, and 136 biotype II), and 31 A. punctata ssp. punctata strains. Among the anaerogenous aeromonads, we found A. hydrophila
ssp. anaerogenes biotype I 24 times, while the remaining 26 (VPT-negative) strains were distributed over A. hydrophila ssp. anaerogenes biotype II and A. punctata ssp. caviae. Since differentiation with the aid of the butane-diol dehydrogenase reaction could not be performed in all strains (cf. page 107), we are able to state only that at least 12 strains belonged to A. punctata ssp. caviae, and at least five strains belonged to A. hydrophila ssp. anaerogenes biotype II. The indole test was negative in 19 Aeromonas strains (ten A. hydrophila ssp. hydrophila biotype I; three A. hydrophila ssp. hydrophila biotype II; three A. hydrophila ssp. anaerogenes biotype I; and three anaerogenous VPT-negative strains). In two strains of A. hydrophila ssp. hydrophila biotype I, production of gas from glycerin at 20°C commenced only after eight and ten days, respectively, but at that time was distinct. Demonstration of arginine dehydrogenase, too, was possible in some strains only after more than a week; this reaction was not forthcoming only in rare cases. Although the utilization of sodium malonate was not tested on a regular basis, we found four strains of A. hydrophila ssp. hydrophila (three of them being biotype I, and one, biotype II) producing an alkaline reaction in malonate medium (after Shaw and Clarke) at 30°C within 24 hours. Two strains of biotype I isolated from various organs of a diseased carp exhibited production of both acid and gas from arabinose, cellobiose and sorbitol after 24 hours, and from lactose after seven days.

In the case of the Enterobacteriaceae isolated, we detected members of the genera Citrobacter, Enterobacter, Hafnia, Klebsiella and Escherichia (cf. Table 2). Among the Citrobacter strains, we found C. freundii as well as C. intermedius. Five of the seven E. coli strains did not split lactose; one did split lactose after a certain period of time. Twelve isolates were,
in fact, identified as Enterobacteriaceae, but could not, on first approach, be assigned to a genus. — The pseudomonads were not differentiated further.

Only in a few diseased fishes did the bacteriological investigation yield no findings or no findings worth mentioning. Among these fishes were three carp of the same provenience, which exhibited an infectious inflammation of the air bladder (early stage). Diagnosis was made on the basis of macroscopic and histological anatomical changes. The primary infectious agent of this particular disease is a virus (Ahne, 1973). In more advanced stages of this disease, we were able to demonstrate a secondary bacterial infection, involving—in addition to various Aeromonas species, subspecies and biotypes—also pseudomonads and Enterobacteriaceae in isolated instances. In two cases of a disease associated with purulent inflammation of the air bladder of cyprinids, the bacteriological results, however, deviated to the extent that pure cultures of bacteria were obtained in both cases from the purulent matter and the organs. In one carp, we isolated exclusively A. hydrophila ssp. anaerogenes biotype I from the purulent matter of the air bladder as well as from the cardiac blood and the kidneys; and in one grass fish, we detected only fluorescent pseudomonads as bacterial agents. In the case of the latter, we were dealing with P. fluorescens biovar B, as was demonstrated by differentiating the strains isolated from Ctenopharyngodon idella performed at Schubert's request and carried out at his institute\(^1\) (personal communication). Bacteriological findings similar to those seen in the air-bladder inflammation of cyprinids were obtained by us in diseased carp suffering from infectious dropsy with edemata and ascites. As a

\(^1\)Cf. the footnote on page 107 [page 15 of this Translation].
characteristic example, we may present the results of an investigation of two carp of the same provenience delivered to us at the same time. We isolated a total of 60 bacterial strains from the cardiac blood, the spleen and the kidney of the two fishes; 56 of these strains were aeromonads of the "hydrophila-punctata group," while the remaining four were Enterobacteriaceae of the genus Citrobacter. Twenty-seven of the Aeromonas strains were found to be A. hydrophila ssp. hydrophila biotype I; 17, A. hydrophila ssp. hydrophila biotype II; three, A. hydrophila ssp. anaerogenes biotype I; five, A. punctata ssp. punctata; and four were found to be A. punctata ssp. caviae (Heuschmann-Brunner, 1971). On the other hand, a further case of infectious dropsy of carp, associated with pyosis of the peritoneum, was distinguished by the sole occurrence of A. hydrophila ssp. hydrophila biotype I in both the purulent matter and the organs examined. All A. hydrophila strains isolated from the purulent matter behaved in completely uniform fashion in the biochemical investigation including the testing of the ability to split lactose, arabinose, cellobiose and sorbitol. In the three afore-mentioned cases of purulent disease of cyprinids we are, thus, permitted to speak of bacterial mono-infection.

In contrast to A. salmonicida, the aeromonads of the "hydrophila-punctata group" appear relatively rarely as pyogenic agents in fishes. That finding may well be due to the formation of a substance toxic for leukocytes (Caselitz and Krebs, 1961) or of two leukocidal substances (Scholz, 1973) demonstrated in A. hydrophila strains. The action of these toxins, to be sure, has been tested only in leukocytes of man and certain [other] mammals.

As in the majority of carp affected with viral diseases, most of the other fishes investigated exhibited mixed infections involving aeromonads,
pseudomonads and Enterobacteriaceae. As a rule, aeromonads of the "hydro-
phila-punctata group" were represented most frequently in these mixed infec-
tions. The exception was found in eel fry (four individuals) obtained shortly
before investigation from an eel breeding farm. In their case, we isolated
chiefly pseudomonads, a smaller number of aeromonads, and a few vibrios from
the organs. Since these young eels exhibited severe infestation of the gut
with the flagellate Hexamita, and that parasite is able to penetrate the
intestinal wall, Hexamita may well be regarded as the "advance party" for
bacterial infection. Among the diseased fishes sent in to the institute,
there were several salmonids with inflammation of the gut. In the case of
these animals, we examined additional mucus specimens taken from the affected
sections of the gut. In most instances, we found good agreement between
the composition of the microflora of the gut and that of the internal organs,
suggesting the occurrence of bacterial dissemination from the gut. In se-
veral fishes exhibiting defects of the skin and the fins or lesions of the
gills, we were permitted to assume that a non-specific bacterial infection
of the organs had occurred, because the dermal epithelium and the gill api-
thelium no longer offered adequate protection against penetration of bacteria.

In particular cases of disease, we used several of the Aeromonas isolated
from the fish organs for testing their pathogenicity in healthy carp by means
of artificial infection. For the purpose of comparison, we included in these
investigations also Aeromonas strains isolated from apparently healthy fishes.
However, before we discuss these investigations, we wish to outline similar
experimental work done earlier, using—at Schubert's suggestion—defined
strains from his collection of aeromonads in carp.

The technique of investigation did not correspond fully to the one used
by us previously, but was modified, following Schubert (1960; 1962; 1964; 1967a),
to the extent that we used for injection exclusively bouillon cultures
incubated for 24 hours at 22°C instead of the bacterial material rinsed
down from the agar medium. In preliminary experiments, we determined the
injection dose optimal for differentiation of highly pathogenic, less
pathogenic, and non-pathogenic Aeromonas strains; we found that dose to
be 0.5 mL bouillon culture per 100 g body weight of two-year-old carp. The
injection was administered intraperitoneally, since the dorsal lymph space
occasionally could not accommodate the quantity of liquid injected, and we
wished to work with exact doses. The two-year-old, slightly scaled shining
(or mirror) carp serving as experimental animals had been obtained from the
Department of Fish Husbandry of our Institute, and were genetically as uni-
form as possible. We tested eight A. hydrophila ssp. hydrophila and eight
A. punctata ssp. punctata strains from Schubert's collection for pathogeni-
city in fishes. In addition, we used two A. punctata ssp. punctata strains
(NCMB 73 and NCMB 74), which we had received from the Institute of Hygiene,
in Bonn, in connection with previous work. These two strains had originally
been isolated by Schaeperclaus from dropsy-diseased carp and forwarded to
the National Collection of Marine Bacteria, in Aberdeen. Two A. punctata
strains denoted "RT" provided by Schubert had been isolated from the hind-
gut of frog (Rana temporaria) taken from a pond. The remaining A. punctata
as well as all A. hydrophila strains had been isolated from water and waste
water specimens.

The injected carp were kept in aquaria with abundant aeration and a rather
low rate of water flow. The temperature of the water normally was 10 to 11°C;
for the purpose of comparison, that temperature was increased to 14 to 15°C
by means of heating. Initially, we used four carp for each experiment. If
one or several experimental animals died already within the first week, we
injected two additional carp, in order to be able to assess the pathogenicity
of the strain in question in an adequate number of fishes. The period of
observation usually covered three weeks, since occurrence of death was not
expected after that period of time. All animals—i.e. not only those that
had died in the course of the experimental test—were subjected to autopsy.
Several of our experiments aimed at determining pathogenicity are summarized
in Tables 3 and 4.

As in Schubert's (1960; 1964) pathogenicity tests, we evaluated a given
test as positive if at least fifty per cent of the experimental animals had
died exhibiting the characteristic manifestations of the disease. That was
the case in _A. hydrophila_ strains No. 3, 4/V and 2/1 as well as in _A. puncta-
tata_ strains NCMB 73 and RT 1/6a (Tables 3 and 4). The highly pathogenic
strains _A. hydrophila_ 3 and 4/V and _A. punctata_ RT 1/6a killed the experimental
animals, as a rule, already after two to three days. In some experiments,
only some of the carp died, while the other ones became ill to a considerably
lesser degree and survived the infection. Since we must assume that the
states of immunity of the individual fishes were not identical, the differ-
ent courses of these pathogenicity tests would have to be attributed, in the
first instance, to these differences in immunity. The manifestations of
this disease usually consisted of pronounced reddening of the ventral side,
edemata of the skin and the internal organs (with distension of the abdomen),
peritonitis, hepatitis, ascites, inflammation of the gut (above all, of the
hindgut), and petechiae in the wall of the air bladder. At autopsy, the
majority of the experimental animals exhibited adhesions of the peritoneum
as the result of peritonitis. This particular finding may have been caused
Table 3 - Experimental work aimed at determining the pathogenicity of different strains of *A. hydrophila* ssp. *hydrophila* Biotype 1 (partial results). Key: 1, *A. hydrophila* strains; 2, Experimental animals (u., and); 3, Duration of experiment, in days; 4, Manner of death; 5, Observations made in the living fishes, and pathological findings made at autopsy; 6, getötet, killed; verendet, died; 7, Transitory loss of appetite; no findings worth mentioning at autopsy; 8, Reddening of the skin; edema of the skin and the internal organs; peritonitis; hepatitis; in part, ascites; inflammation of the gut; isolated petechiae; 9, In poor health, but no signs of an acute process; 10, Reddening of the skin; edema of the skin and the internal organs; peritonitis; hepatitis; ascites; inflammation of the gut; isolated petechiae; 11, After short, light illness, in part, isolated petechiae; 12, Reddening of the skin; edema; peritonitis; hepatitis; in part, inflammation of the gut; petechiae; 13, After light illness, no findings worth mentioning; 14, Reddening of the skin; edema; peritonitis; hepatitis; inflammation of the gut; 15, Two of these fishes were ill and recovered gradually; three overcame the infection without discernible signs of illness.

<table>
<thead>
<tr>
<th>1/1 III</th>
<th>2/1</th>
<th>2/1</th>
<th>2/1</th>
<th>1/1 II</th>
<th>4/V</th>
<th>1</th>
<th>4/V</th>
<th>4/V</th>
<th>5/3</th>
<th>5/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3, 4</td>
<td>1 u. 2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>2,3,4,5</td>
<td>6</td>
<td>1</td>
<td>2,3,4</td>
</tr>
<tr>
<td>30–60</td>
<td>6</td>
<td>8</td>
<td>13</td>
<td>21</td>
<td>31</td>
<td>2</td>
<td>3</td>
<td>60</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>getötet</td>
<td>verendet</td>
<td>verendet</td>
<td>verendet</td>
<td>getötet</td>
<td>getötet</td>
<td>verendet</td>
<td>verendet</td>
<td>verendet</td>
<td>getötet</td>
<td>getötet</td>
</tr>
<tr>
<td>vorübergehend Freßunlust, kein nennenswerter Sektionsbefund</td>
<td>Hautrötung, Ödeme an Haut und Eingeweideorganen, Peritonitis</td>
<td>Hepatitis, z. T. Ascites, Darmentzündung, vereinzelt Petechien</td>
<td>kränklich, aber keine Anzeichen eines akuten Prozesses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/V</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>verendet</td>
<td>verendet</td>
<td>verendet</td>
<td>verendet</td>
<td>getötet</td>
<td>getötet</td>
<td>verendet</td>
<td>verendet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13, nach leichter Erkrankung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>z. T. noch vereinzelt Petechien</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15, zwei dieser Fische waren erkrankt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>und erhellen sich langsam, drei überstanden die Infektion ohne erkennbare Erkrankung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4 - Experimental work aimed at determining the pathogenicity of different strains of A. punctata ssp. punctata (partial results). Key:
1, A. punctata strains; 2, Experimental animals (u., and); 3, Duration of experiment, in days; 4, Manner of death; 5, Observations made in the living fishes, and pathological findings made at autopsy; 6, getötet, killed; verendet, died; 7, Reddening of the skin; edemate of the skin and the internal organs; peritonitis; in part, hepatitis and ascites as well as inflammation of the hindgut, and petechiae on the air bladder; 8, Reddening of the skin; edemata; exophthalmos; peritonitis; inflammation of the hindgut; 9, After light illness, isolated petechiae; 10, Reddening of the skin; edemata of the skin and the internal organs; peritonitis; in part, ascites; severe inflammation of the gut; 11, Reddening of the skin; edemate; peritonitis; severe inflammation of the gut with sanguineous discharge; 12, After light illness, in part, still petechiae; 13, No discernible disease; no findings worth mentioning; 14, Transitory loss of appetite; no findings worth mentioning; 15, No discernible disease; no findings worth mentioning.

<table>
<thead>
<tr>
<th>Stämme</th>
<th>Ver-</th>
<th>Ver-</th>
<th>Todes-</th>
<th>Beobachtungen intra vitam und pathologisch-anatomischer Befund</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>souch-</td>
<td>souch-</td>
<td>art</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tiere</td>
<td>dauer in</td>
<td>Tagen</td>
<td></td>
</tr>
<tr>
<td>NCMB 73</td>
<td>1</td>
<td>6</td>
<td>verendet</td>
<td>Hautröntung, Odeme an Haut und Eingeweideorganen, Peritonitis, z. T.</td>
</tr>
<tr>
<td>NCMB 73</td>
<td>2 u. 3</td>
<td>7</td>
<td>verendet</td>
<td>Hepatitis und Ascites sowie Entzündung des Enddarms und Petechien an der Schwimmblase</td>
</tr>
<tr>
<td>NCMB 73</td>
<td>4 u. 5</td>
<td>8</td>
<td>verendet</td>
<td>Hautröntung, Odeme, Exophthalmus, Peritonitis, Entzündung des Enddarms</td>
</tr>
<tr>
<td>NCMB 73</td>
<td>6</td>
<td>9</td>
<td>verendet</td>
<td>nach leichter Erkrankung, noch verbreitet Petechien</td>
</tr>
<tr>
<td>NCMB 74</td>
<td>1</td>
<td>8</td>
<td>verendet</td>
<td>Haarröntung, Odeme, Peritonitis, starke Darmentzündung mit blutigem Ausfluß</td>
</tr>
<tr>
<td>NCMB 74</td>
<td>2 u. 3</td>
<td>21</td>
<td>getötet</td>
<td>nach leichter Erkrankung, noch Petechien</td>
</tr>
<tr>
<td>NCMB 74</td>
<td>4, 5, 6</td>
<td>22</td>
<td>getötet</td>
<td>keine erkennbare Erkrankung, kein nennenswerter Befund</td>
</tr>
<tr>
<td>RT 1/6 a</td>
<td>1, 2, 3</td>
<td>2</td>
<td>verendet</td>
<td>vorübergehend Freßunlust, kein nennenswerter Befund</td>
</tr>
<tr>
<td>RT 1/6 a</td>
<td>4</td>
<td>3</td>
<td>verendet</td>
<td>keine erkennbare Erkrankung, kein nennenswerter Befund</td>
</tr>
<tr>
<td>RT 1/6 a</td>
<td>5 u. 6</td>
<td>6</td>
<td>verendet</td>
<td>keine erkennbare Erkrankung, kein nennenswerter Befund</td>
</tr>
</tbody>
</table>
by the injection of bacterial material (peritonitis represented the most frequent pathological manifestation seen in the dead fishes) or may also have been due to an earlier incident. Adhesions of the peritoneum are very frequently found in two-year-old carp healthy with respect to both appearance and provenience, and suggest that peritonitis represents a frequent disease in juvenile carp (cf. page 106). For that reason, these particular findings have not been included in the Tables.

The highly pathogenic strains *A. hydrophila* 3 and *A. punctata* RT 1/6a were just as effective when injected in half doses (viz. 0.25 mL bouillon culture per 100 g body weight). In the case of the non-pathogenic strains, doubling of the injected dose and increasing the water temperature by 4 to 5°C did not change the negative result. A repetition of these pathogenicity tests, using both pathogenic and non-pathogenic *A. hydrophila* and *A. punctata* strains, carried out six later using new carp material from our fish farm demonstrated the reproducibility of our results. — The fish-pathogenic *A. punctata* strain NCMB 73 has been described by Schubert (1964) as pathogenic also for frogs. However, the latter author [the German wording could also mean, the present author; Transl.] was not able to induce disease in frogs by means of intramuscular injection of strain *A. punctata* RT 1/6a pathogenic for carp. That finding may be explained by the fact that the incubation temperature of the bouillon culture used for injecting frogs was higher than is conducive for production of toxin by this punctata strain (cf. pages 120 and 121). The *A. punctata* strains K 2232/1 and WC 30 were non-pathogenic for fishes as well as for frogs. It would be desirable to perform comparative investigations regarding the pathogenicity of *Aeromonas* strains of the "hydrophila-punctata group" in a number of different animal species.
in parallel experiments, i.e. at one location, at the same time, using the same bacterial culture, and, if possible, using the same technique.

Anaerogenous aeromonads of the "hydrophila-punctata group" from Schubert's collection were also tested for their pathogenicity in carp. That investigation included 18 strains of *A. hydrophila* ssp. *anaerogenes* (biotype I and biotype II) isolated from waste water, and five strains of *A. punctata* ssp. *caviae*. None of the strains tested turned out to be pathogenic for carp (in accordance with the definition outlined further above). However, one carp injected with *A. hydrophila* ssp. *anaerogenes* biotype I (Strain 028) exhibited, at autopsy at the conclusion of the experiment, suppurative foci in the kidney, which probably were attributable to the injection of that material. Higher doses might well have demonstrated that strain to be pathogenic for fish. In frogs, Schubert (1964a) was unable to demonstrate pathogenicity of anaerogenous representatives of the "hydrophila-punctata group" by means of intramuscular injection.

Corresponding pathogenicity tests were performed using aeromonads of the "hydrophila-punctata group" isolated from the organs of diseased fishes. In these tests, only a part of the *Aeromonas* strains tested turned out to be pathogenic. Those strains that had been isolated in cases of suppurative diseases with bacterial mono-infection of cyprinids (cf. pages 111 and 112) behaved uniformly in the animal experiment, and, following intraperitoneal injection into healthy carp, caused suppurative peritonitis in the experimental animals. Pathological changes of the air bladder of *A. hydrophila* ssp. *anaerogenes* biotype I and *P. fluorescens* biovar B isolated from fishes with suppurative inflammation of the air bladder (cf. page 111) could not be observed. Pathogenic strains of the *A. hydrophila* ssp. *hydrophila* biotype I
were encountered frequently in diseased fishes. Six of 12 *A. hydrophila* strains isolated from the liver, spleen and kidney of a carp suffering from infectious dropsy with edemata and ascites were highly pathogenic; two were less pathogenic; and the remaining four were non-pathogenic. The pathogenic strains isolated from a given organ differed, in part, in their ability to split lactose, arabinose, cellobiose and sorbitol. An even greater predominance of pathogenic over non-pathogenic strains of *A. hydrophila* ssp. *hydrophila* biotype I was found by us in recently imported young Florida softshell (*Trionyx ferox*) suffering from enteritis, edemata, and ascites. The majority of the microorganisms isolated turned out to be highly pathogenic for carp; apparently, these microorganisms were responsible for the severe disease of these reptiles. We are unable to make any statement regarding the pathogenicity of *A. hydrophila* ssp. *hydrophila* biotype II for fishes, since that biotype was put aside in the animal experiments in favor of biotype I. The strains of *A. punctata* ssp. *punctata* isolated from diseased fishes exhibited no, or only very limited, pathogenicity in our infection experiments. The anaerogenous aeromonads of the "*hydrophila-punctata* group" were not tested for their pathogenicity following the negative results obtained by Schübert using strains from his collection (cf. further above)—with the exception of *A. hydrophila* ssp. *anaerogenes* biotype I isolated from a carp suffering from suppurative inflammation of the air bladder. The isolates obtained from that fish were pathogenic, and led in the experiment—as already mentioned further above—to suppurative peritonitis. Schaeperclaus, too, observed occasionally infections with anaerogenous aeromonads of the "*hydrophila-punctata* group in carp (according to Matthes, 1964), and Conroy (1964) described *A. punctata* ssp. *caviae* under the name of *A. formicans*
as agent causing tail rot in goldfish (*Carassius auratus*). That strain, forwarded by Conroy to the National Collection of Marine Bacteria (NCMB 882), was included by us in our pathogenicity tests in carp, but turned out to be non-pathogenic.

Pathogenic aeromonads of the "*hydrophila-punctata* group" are not restricted to diseased macroorganisms, but may be found also in fishes showing no pathological changes as well as in surface water (sewage). The fish-pathogenic strains of *A. hydrophila* ssp. *hydrophila* from Schubert's collection, for example, were isolated from water samples (cf. page 113). The infective pressure exerted on the fish population of a given body of water can be estimated only with difficulty, since the aeromonads of the "*hydrophila-punctata* group" represent microorganisms of highly varying pathogenicity. Investigations by Schubert (1975) and his coworkers have demonstrated that the anaerogenous aeromonads exceed by far the aerogenous ones with respect to numbers. In polysaprobic running waters, the ratio of anaerogenous to aerogenous aeromonads amounts, on average, to about 70:30. According to our pathogenicity tests with anaerogenous *Aeromonas* strains of the "*hydrophila-punctata* group" in carp and those performed by Schubert in frogs (cf. page 116), these microorganisms appear to possess in the afore-mentioned animals a lesser importance as potential pathogens than do the aerogenous aeromonads. Whether that is valid also for other fishes and amphibians can as yet not be resolved, but is probable. In heavily contaminated running waters, the *Aeromonas* number usually amounts to several thousand to > ten thousand cells per milliliter, suggesting a four-digit value for aerogenous aeromonads per milliliter water. In α- and β-mesosaprobic brooks and rivers, the *Aeromonas* numbers, as a rule, are lower by a power of ten; the relative share of aerogenous members of the
"hydrophila-punctata group," however, is greater in the latter waters. Observations of that kind should be made before evaluating the bacterial flora of healthy and diseased freshwater fishes.

Pathogenicity of aeromonads depends on their ability to produce toxins. Using bacteria-free filtrates of bouillon cultures of A. punctata (or of aerogenous aeromonads of the "hydrophila-punctata group," respectively), Schaeperclaus (1939) was able, by means of intraperitoneal injection of these filtrates—to induce manifestations in healthy carp resembling those seen following injection of the bacteria. Caselitz et al. (1960; 1961; 1962; Caselitz, 1966) were able to demonstrate various toxins in A. hydrophila strains. One such toxin, viz. leukocidin, has already been mentioned further above (page 112). In addition, a filtrable hemolysin, which attacked the erythrocytes of rabbits, guinea pigs, and man; a toxin lethal for albino mice; as well as a toxin having a necrotizing action in the animal experiment using guinea pigs and rabbits were demonstrated. It appears that different toxins produced by the aeromonads are affecting fishes. In our investigations, strains of A. hydrophila ssp. hydrophila biotype I highly pathogenic for carp exhibited on carp-blood agar at 30°C occasionally only slow hemolysis, while strains of the same species and subspecies and the same biotype producing hemolysis both more rapidly and more effectively on that medium occasionally were non-pathogenic for carp following intraperitoneal injection. Lobountzov and Roudikov (1968) believed to have found, in culture filtrates of A. punctata (or of aerogenous members of the "hydrophila-punctata group," respectively), a certain relationship between the hemolyzing action on sheep erythrocytes, the cytotoxic effect in tissue cultures, and the lethal action in both albino mice and carp. The question whether they were dealing
with one toxin or several toxic substances remained unresolved. The temperature optimum for growth and production of toxin was found at 25°C in the majority of strains (including those isolated from dropsy-diseased carp); however, a temperature of 37°C appeared to be more suited for some strains.

On the basis of the occurrence of A. hydrophila as agent causing enteritis in man (Caselitz, 1966; Helm and Stille, 1970; among other authors), in dog (Caselitz, 1966), and in other warm-blooded animals, we are permitted to conclude that an enterotoxin is being produced. By means of experimental investigations using rabbit intestines, Sanyal et al. (1975) demonstrated the enteropathogenicity of 12 of 14 A. hydrophila strains of various proveniences. On the basis of the fact that A. hydrophila causes gastro-enteritis in man in countries where raw fish is eaten, the suggestion arises that aeromonads multiply in the dead fish body (Heuschmann-Brunner, 1970a), with enterotoxic substances possibly present undergoing enrichment. Recently, Fritsche et al. (1975) have even reported a case of acute gastro-enteritis caused by A. punctata ssp. caviae in man, suggesting that enterotoxin is produced also by these anaerogenous microorganisms. From the point of view of comparative pathology, it is of interest that carp injected intraperitoneally or intralymphally with bouillon culture of A. hydrophila ssp. hydrophila or of A. punctata ssp. punctata occasionally became diseased with enteritis. Following intraperitoneal injection of A. punctata, Tomasec and Winterhalter (1955) found toxic necrobiosis in the gut of carp, and purulent or fibropurulent inflammation of the mucosa. — In investigations performed by Caselitz et al. at the Institute of Bacteriology of the [Hamburg-] Altona General Hospital, strains of A. hydrophila ssp. hydrophila biotype I, which we had isolated from diseased fishes, did not differ in their production of toxin from A. hydrophila strains of human provenience. For that reason,
it is not surprising that several *A. hydrophila* strains isolated from human material (kindly made available to us by Professor Dr. F.H. Caselitz) exhibited distinct pathogenicity in fish. *A. punctata* ssp. *punctata* strains from Schubert's collection were also investigated regarding their production of toxins by Caselitz and his coworkers. It was found that these strains produce a filterable hemolysin at 22°C, but—in contrast to *A. hydrophila* ssp. *hydrophila*—were only poorly able or not able at all to produce hemolysin at 37°C. (Caselitz, personal communication).

Following infection of the fishes, *A. hydrophila* and *A. punctata* were found to elicit immune reactions in the host organism, leading to the formation of specific antibodies. Agglutinins (Pliszka, 1939; Schaeperclaus and Mann, 1939; among other authors) and antitoxins (Schaeperclaus, 1967) are produced by the fishes, leading to—at least partial—immunization against these microorganisms, which are not uniform in their antigen structure even within a given species or subspecies. In sewage-polluted waters, with their massive presence of aeromonads, immune protection due to immunity will be of particular importance for fishes.

**Discussion**

The aeromonads of the "*hydrophila-punctata* group" play a certain role in many areas, including the biology and hygiene of waters and waste waters, human and veterinary medicine as well as in the bacteriology and pathology of freshwater fishes. *A. hydrophila* ssp. *hydrophila* biotype I possesses the greatest importance among these microorganisms, since it is most active in biochemical terms and, at the same time, is encountered most frequently as agent causing infections in both poikilothermic and homoiothermic vertebrates including man. Treatment of fishes at risk in pond farms with antibiotics
for preventing infections by aeromonads of the "hydrophila-punctata group," thus, represents a justifiable procedure. There exists the risk of producing microorganisms resistant to antibiotics—microorganisms that may get into the water, multiply there, and may be able to transfer their resistance to other microorganisms. Our investigations covering many years have demonstrated that freshwater fishes are threatened by members of the "hydrophila-punctata group" after preceding injury or lesion of the fish organism. In that connection we must give consideration, in the first instance, to certain viral infections like spring viremia and infectious inflammation of the air bladder of cyprinids. Control of these diseases must be directed more strongly against the viral infections by means of preventive measures than against the secondary bacterial infections. A healthy fish is able to protect itself in its habitat against aeromonads.

The taxonomy of the aeromonads elaborated by Schubert (1967; 1969; 1974) meets the requirements of medical microbiology. Precise terminology of these pathogenic agents is advantageous also in the investigation and evaluation of infectious fish diseases. Aeromonas infections must be regarded differently depending on whether they are caused by a certain species and subspecies or a special biotype, respectively, or by several members of the "hydrophila-punctata group" differing in biochemical behavior. Even if the taxonomic system elaborated by Schubert cannot be regarded as being definite, its use has contributed to the expansion of our knowledge of the aeromonads of the "hydrophila-punctata group" in freshwater fishes.

Continuation of investigations dealing with the different aspects of pathogenicity of aeromonads—in particular, of A. hydrophila ssp. hydrophila and A. punctata ssp. punctata—is desirable. Future studies of the toxins of aeromonads and their antigenic action should make use to an increasing
extent of fishes and amphibians (perhaps, the axolotl) as test organisms, in addition to homoiothermic experimental animals. Comparative studies at different temperatures and using bacterial cultures incubated over different periods of time are of importance for all investigations of Aeromonas toxins and antitoxins.

Acknowledgements - I am greatly indebted to Professor R.H.W. Schubert, M.D. (Department of General and Environmental Hygiene at the Center of Hygiene, Frankfurt a. M.) and Professor F.H. Caselitz, M.D. (Department of Bacteriology, General Hospital, Hamburg-Altona) for advice and support.
Bibliography


<table>
<thead>
<tr>
<th>Page</th>
<th>Reference</th>
</tr>
</thead>
</table>

**Translation of foreign-language titles**

1. Cell cultures of various freshwater-teleosteic tissues, and investigations regarding the etiology of inflammation of the air bladder of carp.
2. Recent findings regarding infectious dropsy of carp.
3. Contribution to the spotting disease of pike.
5. Hemolysin studies with *Aeromonas* strains.
6. Investigations of *Aeromonas* strains regarding their action on plasma, fibrin, leukocytes, hyaluronic acid, and collagen.

**Address of the author:**

7. Zoopathogenicity of *Aeromonas* strains.
8. Infectious diseases in trout caused by bacteria.
11. Acute gastroenteritis caused by *Aeromonas hydrophila*.
12. Contribution to the question regarding the agent causing infectious dropsy of carp.
15. Remarks from the point of view of bacteriology regarding the theme of "infectious dropsy of carp."
16. Differentiation between *Aeromonas salmonicida* and the aeromonads of the "hydrophila-punctata group."
17. The intestinal flora of frogs in its dependence on the habitat.
20. Intestinal bacteria of fishes from the point of view of the hygienic evaluation of drinking water.
22. Investigations of the agglutinins of carp.
23. Further investigations of the immune reactions and phagocytosis in carp.
26. Fish diseases.
27. Problems of carp immunity to *Aeromonas punctata*, and questions regarding the antigenic structure of this bacterium.
29. Leukocidal substances of *Aeromonas hydrophila*.
30. Investigations of the characters of the genus *Aeromonas*.
31. The biochemical properties of anaerogenous aeromonads.
32. The biochemical properties of *Aeromonas hydrophila*.
33. Taxonomy of Voges-Proskauer-negative "hydrophila-like" aeromonads.
34. Taxonomy of anaerogenous aeromonads.
35. Taxonomy of the aeromonads.
36. Pathogenicity of aeromonads for man and animal.
37. Occurrence of aeromonads in surface waters.
38. Experimental investigations regarding the factors of importance for the massive outbreak of aeromonads in waste water (sewage).
39. Infra-subspecific taxonomy of Aeromonas hydrophila (CHESTER 1901) STANIER.
40. Aeromonas KLUYVER and VAN NIEL.
41. The relation of aerogenous to anaerogenous aeromonads of the "hydrophila-punctata group" in running waters.
42. Investigations of the etiology of infectious dropsy of carp (Cyprinus carpio L.)
43. A further contribution to the etiology of infectious dropsy of carp.
44. The pathological and morphological picture of carp artificially infected with Pseudomonas punctata.
45. Bacteria of drinking water and surface waters, in particular, of the tapwater of the City of Chemnitz.