The fate of the bacteria *Aeromonas punctata* in the bodies of fish

by L.V. Trofimova, and V.R. Mikryakov

Original Title: O sud'be bakterii *Aeromonas punctata* v organizme ryb


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

Department of Fisheries and Oceans 
Newfoundland Environment Center 
St. John's, Nfld. 
1979

8 pages typescript
The fate of the bacteria *Aeromonas punctata* in the bodies of fish

Biologicheskie nauki

Biological sciences
The fate of the bacteria Aeromonas punctata in the bodies of fish

L.V. Trofimova and V.R. Mikryakov

A comparison was made of the nature of the distribution of inactivated pathogenic bacteria Aeromonas punctata and saprophytic bacteria Hydrogenomonas facilis in the tissues and organs of carp and of the elimination of the products of decomposition of these bacteria. It was shown that organs rich in cells of the reticuloendothelial system have the main role in depositing the introduced bacteria of both species. In the spleen, the localization of tagged carbon per unit weight of tissue is seven times greater from the bacteria of A. punctata than from that of H. facilis. The curves depicting the elimination of the products of decomposition of bacteria A. punctata and H. facilis are identical in shape, but the products of decomposition of A. punctata are eliminated more slowly.

The bacteria Aeromonas punctata cause an infectious disease in fish called aeromonosis, a bacterial form of rubella /3,4/. Studies of the immunological reactions in carp have shown that parenterally introduced A. punctata bacteria undergo phagocytosis and cause the transformation of immunocytes and the synthesis of antibodies /1/. We found no information in the literature as to what happens to these bacteria after phagocytosis. It is important to study this question in order to understand the mechanism of neutralization of pathogenic bacteria in the bodies of fish insofar as the outcome of the interaction between the macro- and microorganism is related to it.
Earlier /2,5,7/, with the aid of the tagged carbon of saprophytic microorganisms, not pathogenic in fish, it was established that the bacteria in the bodies of fish are accumulated by the tissues and break down into carbon dioxide and unidentified organic substances which are eliminated from the bodies of the fish. Some of the tagged bacterial carbon remains in the tissues of the fish for a long time (more than 2 years).

This paper outlines the results of a study of the fate of substances, forming the pathogenic bacteria A. punctata, in the bodies of carp, Cyprinus carpio L. We attempted to find out which tissues and organs of the fish take part in neutralizing A. punctata and whether the pathogenic bacteria undergo parenteral digestion similar to that of saprophytic bacteria.

The experiments were conducted on fingerling carp (20 fish) and yearling carp (10 fish). Carbon-tagged bacteria were obtained by cultivating the bacteria on a fish-peptone broth to which 14C-tagged glucose had been added. They were cultivated in 0.5-litre flasks for 48 hrs. The excess radioactive glucose was removed by numerous rinsings with physiological saline solution. The obtained bacteria were inactivated by heating at a temperature of 70°C for 20 minutes, and were administered to the carp intraabdominally. In order to determine the distribution of the tagged carbon of the bacteria in the tissues, thereby attempting to determine the degree to which the tissues and organs take part in neutralizing A. punctata, 2 billion bacteria were injected into each of the carp, weighing approximately 50 g. This corresponded to 33,000 impulses of radioactivity per minute. When studying the products of decomposition of the tagged bacteria eliminated from the bodies of the fish, 8 billion bacteria with a radioactivity of 120,000 impulses per minute were administered to the carp. The products of bacterial decomposition were determined by the method of V.I. Romanenko and B.A. Flerov /9/, and the distribution of the tagged carbon of the bacteria in the tissue of the carp was determined by the method of V.I. Luk'yanenko and Yu. I. Sorokin /5/.

In order to compare the nature of the neutralization of the pathogenic bacteria in the bodies of the fish with that of the saprophytic bacteria, experiments were set up in which the fish were administered inactivated hydrogen-oxidizing bacteria, Hydrogenomonas facilis, tagged with carbon. The bacteria were tagged by cultivating them on Ruland /transliteration - Tr./ medium, containing radioactive sodium carbonate NaH14CO3.
The degree to which the tissues and organs participate in neutralizing the bacteria was determined by the quantity of $^{14}$C per unit weight of tissue and for the entire organ. The results were processed statistically.

As a result of determining the concentration of $^{14}$C of _A. punctata_ in the tissue of the fish, we established that organs abundant in cells of the reticuloendothelial system are the main depositors of the introduced bacteria (Table 1). We found no

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity of the tagged carbon of bacteria in the tissues and organs of carp (n = 10)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Blood</th>
<th>Serum</th>
<th>Gliot</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Gut wall</th>
<th>Muscles</th>
<th>Brain</th>
<th>Bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. punctata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. facilis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quantity of $^{14}$C, % of introduced carbon per gram of tissue

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Blood</th>
<th>Serum</th>
<th>Gliot</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Gut wall</th>
<th>Muscles</th>
<th>Brain</th>
<th>Bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. punctata</td>
<td>10.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>H. facilis</td>
<td>10.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
</tr>
</tbody>
</table>

Quantity of $^{14}$C, % of considered quantity of radioactivity

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Blood</th>
<th>Serum</th>
<th>Gliot</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Spleen</th>
<th>Gut wall</th>
<th>Muscles</th>
<th>Brain</th>
<th>Bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. punctata</td>
<td>10.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>H. facilis</td>
<td>10.2 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
<td>10.2 ± 0.2</td>
</tr>
</tbody>
</table>

significant difference in the absorption of $^{14}$C of bacteria _A. punctata_ and _H. facilis_ in almost all of the carp tissues and organs studied. Only in the spleen, which is one of the organs which most actively accumulate bacteria, was the localization of the tagged carbon of _A. punctata_ per unit of tissue weight almost 7 times greater than that of _H. facilis_. This confirms the important role of the spleen in neutralizing pathogenic bacteria.
The data in Table 1 show that the quantity of $^{14}\text{C}$ from the bacteria in the blood serum of the fish is considerably greater than the quantity of radioactivity in a clot of blood. In our opinion, this indicates that the tagged carbon of the bacteria enters the blood after digestion of the bacteria by the phagocytizing cells; the leucocytes found in the bloodstream participate very little or not at all in the phagocytosis of the bacteria.

When the proteins of the blood serum were precipitated with a 10% solution of trichloroacetic acid, it was established that the bulk of the tag was bound with the proteins. In the precipitate $0.51 \pm 0.08\%$ of the total amount of radioactivity of the introduced tag was found per gram of precipitate, and in the supernatant, $0.08 \pm 0.01\%$ of the total was found; i.e., the radioactivity of the precipitate was almost 6.6 times greater than that of the supernatant liquid. The distribution of the tagged carbon in the fractions of blood serum was more or less uniform: $34.2\%$ of the total radioactivity found in the blood serum was accounted for by the fraction of albumins, $19.5\%$ - by the fraction of $\alpha$-globulins, $24.4\%$ - by the fraction of $\beta$-globulins and $22\%$ by the fraction of $\gamma$-globulins. The highest tagged-carbon content in the fraction of albumins is explained by the fact that the latter consists of approximately 50% serum proteins. The specific radioactivity of the albumins is lowest, being 0.68, whereas the specific radioactivity of the $\gamma$-globulin equals 1.7. The latter is possibly connected with the inclusion of $^{14}\text{C}$ in the antibodies contained in the fraction of $\gamma$-globulins.

When assessing the importance of the organs and tissues in absorbing the tagged carbon of the bacteria, we established that a considerable amount of the considered radioactivity is localized in the muscles insofar as they comprise almost 40% of the body weight (see Table 1).

The pathogenic bacteria, like the non-pathogenic bacteria, decompose in the body into carbon and unidentified organic

*or tracer. Tr.

**or deposit(ion). Tr.
substances. The shape of the curves characterizing the elimination of the products of decomposition of bacteria A. punctata and H. facilis, in spite of the biological differences of these two species, is identical (Fig.). The quantity of $^{14}$C released in the carbon dioxide is greatest on the 2nd day after the fish have been injected with the tagged bacteria; it decreases in the days that follow, and after 5-6 days, it becomes constant and remains at this level until the end of the study (10 days). The elimination of tagged carbon in the mucus, urine and feces is more uniform, and is less than the amount eliminated in carbon dioxide. Similar data were obtained by B.A. Flerov and
V.I. Romanenko /7/ for crucians while studying the elimination of $^{14}\text{C}$ introduced with bacteria $H. \text{facilis}$. It should be noted that inactivated $A. \text{punctata}$ bacteria are decomposed somewhat more slowly in the bodies of the fish than $H. \text{facilis}$ bacteria. This is indicated by the data on the quantity of $^{14}\text{C}$ eliminated every day and for the 10-day period of the experiment. During the 10 days of the experiment, the fish eliminated 16% of the introduced tagged carbon of bacteria $H. \text{facilis}$ and 11% of the introduced tag of $A. \text{punctata}$. The different rates of elimination of the products of bacterial decomposition may be related to the rate of the metabolic reactions, the rate of phagocytosis and the transfer of the bacteria from the place where they were introduced, and to the differences in absorption by the organs and tissues and in destruction of the bacteria by the enzymes of the cells which absorbed them. The release of carbon dioxide by carp to which $A. \text{punctata}$ had been administered occurred at the same rate as for carp which had been injected with $H. \text{facilis}$ (Table 2). In the tissues, the tagged carbon of bacteria $A. \text{punctata}$

Table 2

The quantity of CO$_2$ released by carp after being injected with bacteria

<table>
<thead>
<tr>
<th>Day of experiment</th>
<th>Quantity of CO$_2$, mg (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H. \text{facilis}$</td>
</tr>
<tr>
<td>1</td>
<td>375 ± 17.9</td>
</tr>
<tr>
<td>2</td>
<td>431 ± 28.1</td>
</tr>
<tr>
<td>3</td>
<td>445 ± 32.4</td>
</tr>
<tr>
<td>4</td>
<td>317 ± 18.7</td>
</tr>
<tr>
<td>5</td>
<td>490 ± 29.6</td>
</tr>
<tr>
<td>6</td>
<td>496 ± 28.8</td>
</tr>
<tr>
<td>7</td>
<td>417 ± 37.4</td>
</tr>
<tr>
<td>8</td>
<td>458 ± 14.1</td>
</tr>
<tr>
<td>9</td>
<td>366 ± 29.6</td>
</tr>
</tbody>
</table>
is not localized in smaller quantities than the tag of bacteria H. facilis. Therefore, we believe that the slower elimination of the products of decomposition of A. punctata can be explained by the slower destruction of the bacteria, acted upon by the enzymes of the cells that absorbed them. Apparently, A. punctata have some mechanisms as pathogenic bacteria which impede their being digested. Possibly, they have a more stable cellular membrane, which has an important role in the sensitivity of microorganisms to the action of intracellular enzymes.

From the data obtained on inactivated bacteria, it may be assumed that native pathogenic A. punctata bacteria in the bodies of fish are also deposited and neutralized by organs which are abundant in cells of the reticuloendothelial system, particularly the spleen and kidneys. Some of the products of bacterial decomposition, formed after the intracellular breakdown of the bacteria, are eliminated from the fish organism. The rate of elimination of these products is related to the biological nature of the bacteria which are introduced.

References


5. Luk'yandenko, V.I. and Sorokin, Yu. I. "Entry rate and distribution of an antigen in tissues of fish (Rutilus rutilus L.)."
Dokl. AN SSSR, 1965, vo. 161, No. 5.

Литература
1. Гончаров Г. Д. Исследование механизма иммунитета рыб к инфекции. В сб.: Обмен веществ и биохимия рыб. М., 1967.
2. Гончаров Г. Д., Романенко В. И., Микрюков В. Р. Исследование механизма иммунитета рыб при помощи "C. Doka. AN CCCP, 1966, т. 171, № 5.
3. Канаев А. Н. Достижения и задачи ветеринарной науки в области патопатологии. Бюл. Всес. ин-та эксперим. ветеринарии, 1975, вып. 20.
6. Романенко В. И., Флеров Б. А. Методика определения элиминации антигена у рыб. Информ. булл. Ин-та биологии внутр. вод. AN CCCP, 1969, № 3.
7. Флеров Б. А., Романенко В. И. Исследование элиминации корпускулярного антигена у рыб. Информ. булл. Ин-та биологии внутр. вод. AN CCCP, 1969, № 3.

Received for publication
4 January 1977

Recommended by the Institute of the Biology of Internal Waters

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISEE
Information seulement