H-ION CONCENTRATION IN THE ALIMENTARY CANAL OF TWO SPECIES OF TELEOSTS, COTTUS SCORPIUS AND ZOARCES VIVIPARUS

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INTRODUCTION

Among the different classes of Pisces, the Teleostei includes the largest number of species and display the greatest diversity of feeding habits. Thus Teleostean forms have stimulated many workers to the study of the alimentary canal, but despite the voluminous work in the field, comparatively little attention has been paid to its physiology.

In the study of H-ion concentration in the alimentary canal of Pisces, various research workers (for example, Herwerden, 1908; Vonk, 1927) have found that the stomach of Teleosts is less acidic than that of Selachians. The pH values of the stomach of teleosts have, so far, been observed to be generally above 3. Bayliss (1935) is the only author who records pH values of 2·4 and 2·7 in the stomach of two specimens of Pleuronectes platessa, although the pH values in the stomach of 23 others varied between 5·5 and 7·6. Determining the reactions in the stomach by Congo paper, Kenyon (1925) found that the pH values in the stomach of several teleosts varied from neutrality to pH 4. Vonk (1927) found that the pH in the stomach of Esox lucius varied from 4·92 to 5·97. Karpevitch and Bokaff (1937) found in Pleuronectes flessus that the pH in the stomach, after the fish had been fed, was, at first, 7·4, but fell to 5·5 towards the end of digestion.

The object of the present work was to determine the H-ion concentration in the alimentary canal of the teleosts with the help of more modern apparatus by using pH electric potentiometer and to correlate the results with the histological structures. Cottus scorpius (L.) and Zoarces viviparus (L.) were the two species selected for the course of this work.

MATERIALS AND METHODS

All specimens used in this work were obtained from Marine Biological Station, Den Helder (Holland), and were kept in the aquarium tanks at the
Laboratory of Comparative Physiology, University of Utrecht, Utrecht (Holland).

The pH measurements were taken in starving and artificially fed fish. The starved fish were kept in clean glass aquarium tanks for 7 days without any food. After this period, food was introduced into the stomach by pushing it gently with a glass rod from the buccal cavity into the stomach. The pH measurements were taken after the food had been in the stomach for 24, 48 and 72 hours.

The fish were killed by pithing, and this operation as well as the removal of the alimentary canal had to be carried out with great care in order to avoid pressing the duodenal fluid into the stomach or vice-versa before the stomach was ligatured at either end. The stomach was then brushed with distilled water to wash away the body fluids from its outer wall in order to determine more correctly the pH of the fluid when it was cut open.

The pH measurements were taken on a Cambridge potentiometer, using quinhydrone electrode.

Observations

The following pH measurements were taken at different locations of the alimentary canal of Cottus scorpius and Zoarces viviparus.

(a) Cottus scorpius

Fish No. 1.—A specimen from an aquarium tank was taken, but no observations were made on its feeding. The stomach was empty. Very little food was found in the posterior part of the intestine (Fig. 1 a).

Fig. 1. pH measurements in the stomach, duodenum and pyloric caecum of Cottus scorpius. The anterior and posterior regions of the stomach are divided by a dotted line. (a) Random specimen, (b) Starved specimen, (c) 24 hours after giving food, (d) 48 hours after giving food, (e) 72 hours after giving food.
Fluid oozing from the middle region of the stomach when it was cut open ........ 7.7

Four different places on the mucosal wall of the anterior portion of the stomach ....... 7.3, 7.3

At 3 different places on the mucosal wall of the posterior region of the stomach .......... 7.5, 7.7

In the duodenum .................. 7.9

In the pyloric cæcum ............... 7.8

Fish No. 2.—A fish was kept without food for 7 days in an aquarium tank containing clear sea-water. No food was found in the alimentary canal after the stravation period (Fig. 1 b).

Fluid (very little) oozing from the middle region of the stomach when it was cut open ........ 7.6

At 4 different places in the anterior region of the stomach .. 7.3, 7.3

At 4 different places in the posterior region of the stomach .. 8.0, 8.2

In the duodenum .................. 8.6

In the pyloric cæcum ............... 8.6

Fish No. 3.—The fish was fed with a meal of small Mytilus edulis with valves open, sliced fish, chopped flesh of Mytilus edulis and a crab. pH measurements were taken after the food had been in the stomach for 24 hours. No food had passed from the stomach into the intestine (Fig. 1 c).

Fluid oozing from the middle region of the stomach after it had been cut open ........ 5.85

On a piece of sliced fish .................. 5.6

At 4 different places in the anterior region of the stomach ............... 5.6, 5.7, 5.7, 5.9

At 4 different places in the posterior region of the stomach .............. 6.7, 6.8, 6.82, 6.9

In the duodenum .................. 7.4

In the pyloric cæcum ............... 7.4

Fish No. 4.—A fish was fed on the same meal as was given to Fish No. 3. pH measurements were taken after the food had been in the stomach for 48 hours. No food had passed into the intestine (Fig. 1 d).
H-Ion Concentration of Cottus scorpius and Zoarces viviparus

**LOCATION**

<table>
<thead>
<tr>
<th>Location Description</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid oozing from the middle region of the stomach when it was cut open</td>
<td>4.4</td>
</tr>
<tr>
<td>On the inside of opened <em>Mytilus edulis</em> after the stomach had been cut open</td>
<td>4.5</td>
</tr>
<tr>
<td>Fluid lying in the depression of the mucosal wall after the removal of <em>Mytilus edulis</em></td>
<td>4.08</td>
</tr>
<tr>
<td>On a piece of sliced fish lying in the anterior region of the stomach (marked ×)</td>
<td>3.6</td>
</tr>
<tr>
<td>At 4 different places in the anterior region of the stomach</td>
<td>4.8, 4.9, 5.1, 5.2</td>
</tr>
<tr>
<td>On a piece of the flesh of the crab lying in the posterior region of the stomach</td>
<td>4.3</td>
</tr>
<tr>
<td>On a piece of sliced fish lying in the posterior region (marked Δ)</td>
<td>4.46</td>
</tr>
<tr>
<td>At 5 different places in the posterior region of the stomach</td>
<td>5.4, 5.6, 5.8, 6.4</td>
</tr>
<tr>
<td>In the duodenum</td>
<td>7.8</td>
</tr>
<tr>
<td>In the pyloric cæcum</td>
<td>7.7</td>
</tr>
</tbody>
</table>

**Fish No. 5.**—A fish was fed on the same meal as Fish No. 3. pH measurements were taken after the food had been in the stomach for 72 hours. A few slices of chopped fish had passed into the intestine (Fig. 1 e).

**LOCATION**

<table>
<thead>
<tr>
<th>Location Description</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid oozing from the middle region of the stomach</td>
<td>4.2</td>
</tr>
<tr>
<td>On a soft piece of the flesh of the crab lying in the anterior region of the stomach</td>
<td>3.42</td>
</tr>
<tr>
<td>At 4 different places in the anterior region of the stomach</td>
<td>4.4, 4.4, 4.4, 4.6</td>
</tr>
<tr>
<td>On the inside of <em>Mytilus edulis</em></td>
<td>4.3</td>
</tr>
<tr>
<td>On the flesh of the crab lying in the posterior region of the stomach</td>
<td>4.5</td>
</tr>
<tr>
<td>At 5 different places in the posterior region of the stomach</td>
<td>4.6, 4.6, 4.8, 5.1</td>
</tr>
<tr>
<td>In the duodenum</td>
<td>7.7</td>
</tr>
<tr>
<td>In the pyloric cæcum</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Fish No. 1.—A fish was kept for 7 days in an aquarium tank. The alimentary canal was found to be devoid of food after the starvation period (Fig. 2 a).

Fish No. 2.—A fish was fed on chopped crab and fish. pH measurements were taken after the food had been in the stomach for 24 hours. No food had passed into the intestine (Fig. 2 b).

Fish No. 3.—A fish was fed on the same meal as fish No. 2. The food remained in the stomach for 48 hours without being passed into the intestine (Fig. 2 c).
Fish No. 4.—A fish was fed with the same meal as Fish No. 2. The food remained in the stomach for 72 hours without being passed into the intestine (Fig. 2d).

**Location**

<table>
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<tbody>
<tr>
<td>(1) Fluid oozing from the stomach</td>
<td>4.8</td>
</tr>
<tr>
<td>(2) On the flesh of fish lying in the middle region of the stomach (marked ×)</td>
<td>4.7</td>
</tr>
<tr>
<td>(3) On the flesh of the crab also lying in the middle region of the stomach</td>
<td>4.95</td>
</tr>
<tr>
<td>(4) At 2 different places in the anterior region of the stomach</td>
<td>4.75, 4.9</td>
</tr>
<tr>
<td>(5) At 3 different places in the posterior region of the stomach</td>
<td>4.9, 5.2, 5.4</td>
</tr>
<tr>
<td>(6) In the duodenum</td>
<td>7.8</td>
</tr>
</tbody>
</table>

**Discussion and Conclusion**

The reactions in the stomachs of the starved specimens of *Cottus scorpius* and *Zoarces viviparus* might have been neutral or alkaline, but were not acid. The neutral or alkaline reactions in the stomach would suggest that either no acid was secreted or that the acid secreted was neutralised. It is not yet known whether secretion of acid in the stomach of any vertebrate is a continuous process or whether it is intermittent. If acid is secreted when no food is present in the stomach, it must be neutralised by either the alkaline gastric mucus or the regurgitation of alkaline intestinal fluids. The gastric mucus in mammals is alkaline and has an acid-binding property (Babkin, 1944, p. 214). Bayliss (1935) suggests the possibility of acid in the stomach of *Pleuronectes platessa* being neutralised by the intestinal fluid. In *C. scorpius* and *Z. viviparus* the possibility of gastric mucus neutralising the acid secretion during starvation is greater than that of intestinal regurgitation, as the opening between the stomach and the intestine is greatly constricted and is also provided with gastro-intestinal fold.

The gastric contents are acid while food is present in the stomach. The rise in acidity is very slow. The secretion of the gastric juice, which is scanty after starvation, increases considerably 24 hours after food is introduced into the stomach. When the food has been in the stomach for a longer time (observations relate to only 72 hours after introduction of food) the gastric juice becomes more acid.

In all the specimens of *Cottus scorpius* and *Zoarces viviparus* examined for pH determinations, it is found that pH values are generally lower in the
anterior region of the stomach than in the posterior region. The average pH values in the anterior region of the stomach of *C. scorpius* as observed after starvation, and for 24, 48 and 72 hours after introduction of food, were 7·36, 5·72, 5·04 and 4·5 respectively; the pH values in the posterior region under the same conditions were 8·28, 6·84, 5·77 and 4·74 respectively. The average pH values in the anterior region of the stomach of *Z. viviparus* under the same conditions were 6·9, 5·8, 5·12 and 4·85 respectively. The pH values in the posterior region were 7·5, 7·0, 5·85 and 5·15 respectively. These differences in pH between the anterior and posterior regions of the stomach indicate that the former is more acidic than the latter.

It seems likely, therefore, that acid is secreted only in the anterior region, the posterior being acidic because it receives it from the anterior region. The two parts are in open communication in the short stomachs of the two species. Secretion of acid in the anterior region is quite in accordance with the histological observations that the gastric glands are found in the anterior region (= corpus) and are absent in the posterior region (= pylorus). As the other histological features of the two regions of the stomach in these two species are similar, it seems likely that the acid in the corpus is secreted by the gastric glands.

In the starved specimens of *C. scorpius* and *Z. viviparvs* the anterior and posterior regions of the stomach also showed differences in pH, suggesting that acid was secreted despite absence of food from the stomach.

Various workers have shown that pepsin in fishes is produced in the corpus and not in the pyloric region (Yung, 1898; Sullivan, 1905; Herwerden, 1908; Beauvalet, 1933). This would mean, therefore, that both acid and pepsin are secreted by the gastric glands in the corpus. The gastric glands in fishes are not differentiated into oxyntic and peptic cells (Edinger, 1877). This has also been confirmed by later workers. My work on these two species has led to, also, similar results. If all the cells of the gastric glands are identical, then they must have the dual function of secreting both acid and pepsin.

On the other hand if the cells are physiologically different, then various chemical stimuli, such as histamine, acetylcholine and pilocarpine should bring about the qualitative change in the gastric juice which, in turn, would depend upon the unequal quantitative activity of two kinds of cells of the gastric glands. Dobreff (1927) found that there is no psychic phase in the gastric secretion in *Scyllium canicula*. He also found that intramuscular injection of pilocarpine, acetylcholine, atropine and histamine had no effect
on the secretion of the gastric juice and thus inferred that the control of gastric secretion must be humoral. Babkin, Chaisson and Friedmann (1935) did not find any evidence of nervous control of the secretion in Raja diaphanus and R. erinacea. Bayliss (1935) injected Pleuronectes platessa with pilocarpine and considered this was followed by increased secretion of pepsin. Although there are conflicting opinions on the factors controlling gastric secretion and more data are required, it may provisionally be suggested that the evidence now available supports the view that both pepsin and acid are secreted by the same cells.

Although in Cottus scorpius some of the food particles had passed from the stomach into the intestine between 48 and 72 hours after introduction of food, the lowest pH observed was 3·42 on a piece of soft flesh in the stomach for 72 hours. In Z. viviparus the lowest pH observed was 5·05 on the wall of the corpus, when the food had been in the stomach for 72 hours. From the pH values observed it seems, therefore, that there must not be much free HCl during gastric digestion.

The free HCl in the gastric juice is generally determined by titrating gastric juice with 0·1 N. NaOH using Topfer's indicator which changes the colour from red to yellow between pH 2·9 and 3·6. Most of the observations on pH determinations in the stomach of teleosts, including the present two species, would suggest that there is little free HCl.

Under the same conditions of artificial feeding in C. scorpius and Z. viviparus the rise of acidity in the former is more than in the latter.

The pH values on the food substances lying in the stomach are generally lower than the values on the underlying stomach wall.

The pH values in the pyloric caeca of Cottus scorpius are nearly the same as those in the duodenum. The duodenum has an alkaline reaction.

Acknowledgement

I acknowledge my indebtedness to Prof. A. D. Hobson, for his advice, freely given, on all occasions and for his guidance throughout my work. My thanks are due to Dr. H. J. Vonk, Director, Laboratory of Comparative Physiology, University of Utrecht (Holland), for extending the facilities of the laboratory.

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