DISTRIBUTION OF IRON IN THE TISSUES OF SOME BIVALVE MOLLUSCS

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ABSTRACT

The quantitative distribution of iron in the tissues has been studied in three species of bivalves. Two of these, Arca granosa and Arca inaequivalvis possess haemoglobin in their blood corpuscles, whereas the third species, Meretrix casta, which has been investigated for comparison, does not possess haemoglobin. The concentration of iron in the digestive diverticula of Arca is considerably higher than in the other tissues and also than that in the digestive gland of Meretrix. The relatively high concentration of iron in the digestive diverticula is similar to that in the haemopoietic organs of vertebrates. This would suggest that the digestive diverticula of Arca have possibly a role similar to that of the haemopoietic organs.

INTRODUCTION

Iron is micro-constituent of all living tissues. Its micro-concentration generally varies from 0.01 to 0.10 mg. per gm. of cell substances (Fearon, 1948). It is also an invariable secondary element in all red-blooded animals. In the marine biosphere the concentration of heavy metals is significantly higher than in the hydrosphere (Cornec, 1919). With notable exceptions, such as copper in haemocyanin and iron in haemoglobin, the trace elements found in many invertebrates have no known function. Vinogradov (1953) has compiled data on the elementary chemical composition of living organisms, but much of the work referred to by him was conducted long ago by experimental techniques which would not be regarded now as valid. Recently, Galtsoff (1964) investigated in Crassostrea virginica (Gmelin) the seasonal variation in iron content. Brooks (1965) investigated the biogeochemistry of trace element uptake by some New Zealand bivalves. Hobden (1967) has studied the iron metabolism in Mytilus edulis. Partly in view of the relative paucity of information on the distribution of iron in marine
animals, the present study was made on the distribution of iron in the tissues of bivalve molluscs, *Arca granosa* (Linne), *Arca inaequivalvis* (Bruguier). A more important justification is that *Arca* is one of the very few bivalves, which have haemoglobin contained in blood corpuscles. The tissues of *Meretrix casta* (Chemnitz), which have no haemoglobin, have also been studied for comparison.

**Material and Methods**

Freshly collected bivalves were cleaned and kept in filtered sea-water for two days before being used, so that the undigested food materials might be eliminated from the intestine.

A stainless steel scalpel was used to cut the adductors of the animals in a sample. The following tissues were isolated: mantle, gills, foot, adductor muscle and digestive diverticula. They were washed well in distilled water and dried between the folds of filter-paper and weighed. For each estimation tissues from eight animals were pooled together. The tissues were digested in H$_2$SO$_4$. An equal quantity of saturated potassium persulphate was added and the mixture left overnight. The solution was then diluted with distilled water and a few drops of 10% sodium tungstate added to precipitate protein. The solution was then filtered and made up to a known volume. Five ml. of 2M potassium thiocyanate was added to 5 ml. of this solution and the optical density at 480 m$\mu$ measured immediately. Standard solution was prepared from freshly prepared solution of ferric ammonium sulphate. Following the suggestion of Collins and Diehl (1959) the glassware other than digestion flasks was not subjected to acid cleaning.

The data relating to iron content in different tissues were statistically tested. The values were found to be significant at 5% level.

**Histochemistry**

Standard histochemical techniques were used to locate iron within the cells and tissues. Tissues were fixed in 10% neutral formalin embedded in wax and cut into sections 10 m$\mu$ thick. Dewaxed sections were treated with 2% sodium ferrocyanide for 5 minutes, then with a mixture of equal volumes of 2% sodium ferrocyanide and 2% hydrochloric acid for 1 hour. This solution was renewed after 30 minutes. Some sections were counterstained with eosin. Final mounting was with Canada balsam.
Iron in the Tissues of Some Bivalve Molluscs

OBSERVATION AND RESULTS

The iron content of the different tissues in the different species investigated is shown in Table I. It will be observed that the tissues of Meretrix casta have distinctly lower iron content than those of Arca. The two species of Arca differ in their iron content. However, this difference is less than that between Arca and Meretrix. Arca granosa has a higher iron content than Arca inaequivalvis. Of the tissues in Arca, the digestive diverticula have the highest iron content, three or four times the quantity found in any other tissue examined. In Meretrix, however, the iron content of digestive diverticula is not more than twice that in any of the other tissue of the animal.

**Table I**

_Distribution of iron in the tissues of some bivalve molluscs_

<table>
<thead>
<tr>
<th>No.</th>
<th>Foot</th>
<th>Ad. muscle</th>
<th>Gill</th>
<th>Mantle</th>
<th>Digestive diverticula</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>μ/g. (wet wt.)</td>
<td>μ/g. (wet wt.)</td>
<td>μ/g. (dry wt.)</td>
</tr>
<tr>
<td>1</td>
<td>A. granosa</td>
<td>27.2 (33.8-40.1)</td>
<td>50.7 (44.2-56.2)</td>
<td>47.8 (43.5-53.1)</td>
<td>27.8 (22.4-32.9)</td>
</tr>
<tr>
<td>2</td>
<td>A. inaequivalvis</td>
<td>31.9 (23.4-42.9)</td>
<td>40.0 (34.2-45.2)</td>
<td>33.1 (29.9-49.9)</td>
<td>28.8 (21.7-40.9)</td>
</tr>
<tr>
<td>3</td>
<td>M. casta</td>
<td>22.6 (19.9-26.1)</td>
<td>27.4 (22.4-32.8)</td>
<td>25.0 (20.8-34.3)</td>
<td>15.5 (10.5-30.3)</td>
</tr>
<tr>
<td>1</td>
<td>A. granosa</td>
<td>165.5</td>
<td>239.0</td>
<td>172.6</td>
<td>226.9</td>
</tr>
<tr>
<td>2</td>
<td>A. inaequivalvis</td>
<td>140.7</td>
<td>196.0</td>
<td>146.7</td>
<td>160.8</td>
</tr>
<tr>
<td>3</td>
<td>M. casta</td>
<td>92.3</td>
<td>137.5</td>
<td>95.7</td>
<td>120.6</td>
</tr>
</tbody>
</table>

Iron in particulate form was present only in the digestive diverticula and no iron could be demonstrated histochemically in the other tissue sections, even after hydrogen peroxide treatment.

The high content of iron in the digestive diverticula of Arca is noteworthy; it is about 154.9 μ/g. wet weight in Arca granosa and 116.4 μ/g. in Arca inaequivalvis. For other tissues the mean value is only 40.9 μ/g. wet weight in Arca granosa and 33.3 μ/g. in Arca inaequivalvis. In Meretrix the iron content of the digestive diverticula is 43.1 μ/g. wet weight and the mean value for the other tissues is 23.9 μ/g.
DISCUSSION

In the species investigated now, the iron content in terms of wet weight was highest in the digestive gland, and lowest in the mantle. The order of increasing iron content was mantle → foot → gills → adductor muscle → digestive diverticula. But considered in terms of dry weight the order was the foot → gills → mantle → adductor muscle → digestive diverticula. This difference in the order of increasing iron content in wet and dry tissues is easily accounted for, as the foot and gills contain more water than the mantle and adductor muscle.

Bloch-Raphael (1939) found iron in the heart body in polychaetes and suggested an haematopoietic function of the heart body, which has been later corroborated by Dales (1965). He also found in the older worm a tendency to have more haematin in the heart body and that the total iron content increases along with haematin index. The haematin which accumulates in the heart body tissue is believed to be a by-product of haem protein synthesis.

The iron content in vertebrate tissues expressed as percentage of total body weight is generally as follows: Liver 0.008%; spleen 0.009% (Fearon, 1948). The iron content of digestive diverticula of Arca expressed as percentage of total body weight is 0.005%. The higher content of iron in the digestive diverticula than in other tissues of Arca is analogous to that in the haemopoietic organs of vertebrates. This would suggest that the digestive diverticula of Arca may have a role analogous to that of the haemopoietic organs, and possibly may contain porphyrin-bound iron.

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