THE CHLORIDE REGULATION OF THE BRACKISH AND FRESH-WATER RACES OF *MESIDOTEA ENTOMON* (L.)

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INTRODUCTION

The Isopod *Mesidotea* (*Chiridothea*) *entomon* occurs as a glacial relict in the Baltic and a few Swedish lakes (Ekman, 1919, 1940) and a closely related form *M. siberica* is found in the Arctic Ocean. According to Bruun (1924) *M. entomon* and *M. siberica* are the same species. That members of an allegedly single species should occur in both fresh and sea water is most unusual. It is clear that a comparative study of the physiology of individuals from the different habitats might be expected to give information about the relation between the various races and the mechanisms involved in the adaptation to fresh water. The evolutionary aspect of this adaptation is of particular interest as the fresh-water habitats of *Mesidotea* in Sweden have only appeared since the end of the last Ice-age.

The only previous work on the osmotic regulation of *Mesidotea* is that of Bogucki (1932), who studied the chemical composition of the haemolymph of *Mesidotea* from the Polish coast. He found that in dilute sea water the chloride concentration of the haemolymph was distinctly greater than that of the medium, but was approximately the same as that of the medium when the latter approached the concentration of pure sea water. His animals could not tolerate transfer to fresh water.

The purpose of the present investigation was to make a comparative study of the haemolymph chloride concentration of animals from fresh and brackish water both in their normal media and in media of various salinities.

MATERIALS AND METHODS

Mesidotea occurs throughout the Baltic and in six Swedish lakes. Specimens were collected from depths of 15–25 m. by the use of a small iron-framed trawl during the months of July and August 1956. Brackish-water animals were caught off Trelleborg in the south Baltic and also near Fågelsundet at the entrance to the Gulf of Bothnia. Fresh-water animals were obtained from Lake Vättern and the Lilla Ullevifjärden, a northern branch of Lake Mälaren. Most of the animals caught were in the size range 20–35 mm.

The specimens were kept in their natural medium or in experimental media made by diluting either Baltic sea water or a stock of previously concentrated

Plymouth sea water. They were fed occasionally on fragments of *Mytilus* or other suitable material.

The animals from the south Baltic were kept at 5° C. All the others were kept at room temperature.

Acclimatization was carried out in a series of stages, several days being allowed in each medium before haemolymph was taken or before the animals were transferred to the next stage.

In order to collect haemolymph the animals were first dried with filter-paper, and a micro-pipette was inserted into the haemocoel through a dorsal intersegmental membrane in the posterior thoracic region. The haemolymph was stored under liquid paraffin in a lacquered watch glass. A jelly-like clot often developed, but it was possible to obtain the serum by squeezing the clot with a needle.

The haemolymph chloride concentration was determined by electrometric titration using the first method of Ramsay, Brown & Croghan (1955). Titrations were carried out in duplicate or triplicate on samples from single animals and also on samples of the medium. The values obtained were compared with those given by the same volume of a known concentration of NaCl. The standard deviation of a series was about ± 1 %. The concentration of the fresh-water media quoted below are only approximate because the accuracy of the method is much less when very dilute solutions are used.

RESULTS

The results are plotted and summarized in Fig. 1.

Trelleborg animals

The mean chloride concentration of the haemolymph was 335 mM./l. The range of variation for seven animals was 315–360 mM./l. The two readings marked with a query on the graph are not included in the above, as it was suspected that these animals had been crushed or otherwise damaged when caught.

The chloride concentration of Trelleborg sea water was 122 mM./l.

Three animals were transferred directly to full strength Plymouth sea water and were still active and apparently normal after a week. In this medium the haemolymph chloride concentration was very close to that of the sea water, but in all more dilute media tested it was definitely higher. It was possible to acclimatize the animals by several stages to approximately 10 % Trelleborg sea water (14 mM./l. Cl), but when twelve animals were transferred to fresh water (Lund tap water, about 1·7 mM./l. Cl) many soon died. After three days all but one were dead or moribund. The haemolymph chloride of two of these moribund animals was measured. The values were 54 and 49 mM./l. Cl, indicating that a very considerable loss of chloride had occurred. Two other moribund animals were then transferred to Trelleborg sea water. After 3 hr. both had fully regained their normal activity and their haemolymph was found to contain 188 and 174 mM./l. Cl respectively. The only animal that was still active on the third day in tap water was moribund by the fifth day. Its haemolymph chloride had fallen to 89 mM./l. This animal was transferred to Trelleborg sea water and had recovered normal activity after 4 hr. On re-sampling the haemolymph a chloride concentration of 195 mM./l. was found. These results indicate that a very rapid active uptake of chloride is possible, but that this mechanism can operate effectively only in media containing

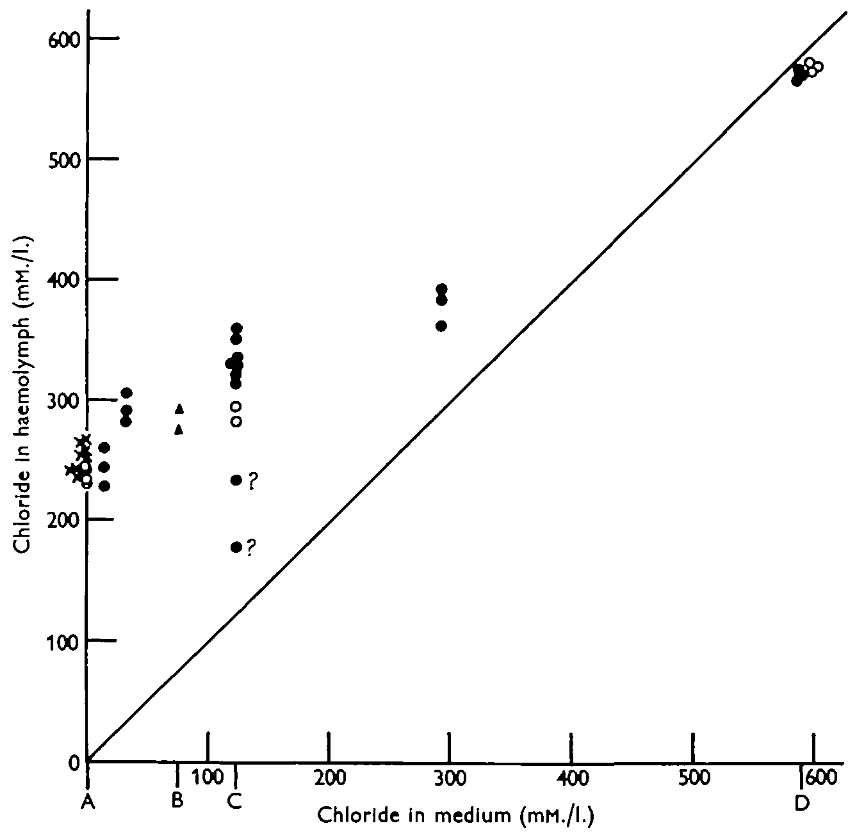

Fig. 1. The relation between the chloride concentration of the haemolymph and that of the medium. Each point represents a sample from a single animal. The diagonal represents equality of concentration. Baltic animals: from Trelleborg, ●; from Fågelsundet, ▲. Freshwater animals: from Vättern, O; from Mälaren, × ; (?see text). A, fresh water; B; Fågelsundet sea water; C, Trelleborg sea water; D, Plymouth sea water.

appreciably more chloride than does fresh water. A very rough estimate of the rate of uptake can be made by taking the average weight to be 0·50 g. and assuming the haemolymph to be 50% of the body weight; this gives a rate of uptake of 6·5–11 μM./animal/hr. or 13–22 μM./g./hr.

Fågelsundet animals

The mean chloride concentration of the haemolymph was 285 mM./l. (two animals, respectively 276 and 294 mM./l. Cl).

The chloride concentration of the Fågelsundet sea water was 75 mM./l. Five animals were transferred directly to fresh water and after 2 days four were dead. The survivor was found to have a haemolymph chloride of only 110 mM./l.

Lake Vättern animals

The mean chloride concentration of four animals was 239 mM./l. (range 234–245 mM./l.)

The Lake Vättern water had a chloride concentration of about 0·2 mM./l. Animals were acclimatized by stages to full strength Plymouth sea water. After 5 days they were still apparently normal and the haemolymph chloride of the three sampled was very close to that of the medium.

Lake Mälaren animals

The mean chloride concentration of the haemolymph was 253 mM./l. (eleven animals, range 237–266 mM./l.).

The chloride concentration of Lake Mälaren water was about 0·5 mM./l.

No further experiments were performed on these animals.

DISCUSSION

The main interest of the present study on *Mesidotea* lies in the information it may give about the evolution of a fresh-water animal from brackish water ancestors. The evolution of *Mesidotea* as an inhabitant of fresh water is of particular interest, since its fresh-water habitats in Sweden are post-glacial and it must therefore have evolved relatively recently. It is very much more restricted in its fresh-water distribution than are the other glacial relicts. In Sweden it is confined to a few lakes across the central lowlands and this fact might also suggest a late origin.

The Baltic *Mesidotea* has evolved as a brackish-water animal, maintaining in dilute sea water the high haemolymph concentration characteristic of brackish-water Crustacea. The animal can be adapted to very dilute media, and occurs even in the northern Baltic, but could not be acclimatized to fresh water—at least, not in the time available for the present study. There is thus a clear physiological distinction between the brackish-water (Baltic) and the fresh-water races. On morphological grounds, Ekman (1919) regarded the form from Vättern as being distinct (*M. entomon* f. *vetterensis*).

The haemolymph chloride of the fresh-water race is also high and is comparable with that found in *Eriocheir sinensis* in fresh water by Scholles (1933) and in the fresh-water race of *Gammarus duebeni* by Beadle & Cragg (1940*b*). The fresh-water race of *Mesidotea* can be adapted to brackish waters and possesses the ability, remarkable in an animal normally passing its entire life in fresh water, to survive in full strength sea water. In saline waters the haemolymph chloride concentration of the fresh-water race is similar to that of the Baltic race.

It appears that the basic osmotic physiology of the two races is remarkably similar. The difference is that the fresh-water race has developed a more effective osmo-regulatory mechanism that enables it to maintain the high haemolymph concentration of the brackish-water race even in pure fresh water. This is similar to what has occurred in the evolution of the fresh-water race of *Gammarus duebeni* (Beadle & Cragg, 1940*b*; Beadle, 1943).

Beadle & Cragg (1940*a*) and Beadle (1943) have regarded a high haemolymph concentration in fresh water as indicating an early stage in the evolution of a fresh-water animal. Potts (1954) points out that this could only be so in animals with low permeabilities. In Crustacea a low permeability would be expected as a result of the presence of a well-developed cuticle. Both the high haemolymph concentration and the ability of the fresh-water *Mesidotea* to survive in full strength sea water may be regarded as primitive features. The physiological evidence is thus in keeping with the other evidence for a recent origin of the fresh-water race.

The nature of the adaptation to fresh water in *Mesidotea* was not studied. It may be due to decreased permeability, to a more effective ion uptake mechanism, or possibly even to the production of a hypotonic urine. The brackish-water race has a powerful active uptake mechanism maintaining the haemolymph concentration hypertonic to brackish waters, and an increase in the efficiency of this might be a factor in the adaptation to fresh water. A comparative study of the permeability and active uptake of the two races would be of considerable interest.

SUMMARY

1. *Mesidotea entomon* (L.) is found in the Baltic and in certain fresh-water lakes in Sweden. It is believed that colonization of fresh water in this region has taken place since the last Ice-age.

2. In the present work animals from brackish and fresh-water habitats have been compared both in respect of the concentration of chloride in their haemolymph and of their ability to survive in media of various salinities.

3. Both fresh-water and Baltic animals have been found able to survive in Plymouth sea water, the concentration of chloride in their haemolymph being close to the concentration of chloride in this medium.

4. Baltic animals could not be acclimatized to fresh water.

5. Animals from both habitats have the same general level of chloride concentration in their haemolymph when acclimatized to dilute sea water.

6. These results are discussed in relation to the evolution of a fresh-water race from a brackish-water race.

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