CHLORIDE REGULATION IN *LYCASTIS INDICA* SOUTHERN*

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Lycastis indica show a tolerance to wide range of salinity of the external medium and are able to thrive in fresh water. This is attributed to their ability to regulate the chloride content of the body fluids. Under experimental conditions, in media of low salinity, the chloride content of the body fluid is correspondingly lowered. The chloride values of the body fluid are found to be always less than those of the media to which they are acclimated. The results have been discussed in the light of previous work on the influence of the external medium on osmotic behaviour. The hypo-regulation of chloride appears to be of adaptive value to the animals enabling survival under heterosmotic conditions.

INTRODUCTION

It is known that nereids exhibit varying degrees of tolerance to dilution of external medium. But a feature noted among them is that members of the same species often differ markedly in osmotic behaviour and such differences have been attributed to the influence of the salinity of the medium. *Nereis diversicolor* collected from different habitats have been shown to differ markedly in the osmotic regulation of their body fluids as inferred from the changes in weight under heterosmotic stress (Schlieper 1929; Beadle 1937; Ellis 1937). The last-mentioned author observed that under identical experimental conditions *N. diversicolor* from Roscoff showed better weight regulatory ability than those collected from Plymouth and Bangor. Schlieper (1929) noted that *N. diversicolor* collected from brackish waters in the neighbourhood of Kiel maintained its internal concentration at a higher level than that of the external medium when subjected to 25% and 50% sea water. It has been suggested by Beadle (1937) that 'the osmoregulatory mechanism of the animals with which Schlieper worked was better developed as a result of continual subjection to water of low salinity'. The results reported above may suggest that the differences in osmotic behaviour of the worms from the two geographical regions, Roscoff and Plymouth, may be

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attributed to what has been called 'physiological adaptation'. Recent work of Gross (1963) on *Hemigrapsus oregonensis* provides evidences for such 'physiological adaptation' resulting in improved osmoregulatory ability. *Lycastis indica* used in the following study is able to thrive in sea water as well as in various dilutions of it and in near fresh-water conditions. But very little is known of the mechanism involved in their ability to survive different degrees of dilution of the external medium. In this context, it is of interest to investigate under experimental conditions, the influence of the salinity of the external medium on the osmotic behaviour of *Lycastis indica*. The object is to find out how acclimation to low salinity conditions contributes to a more efficient regulation of weight and thereby better survival under heterosmotic conditions.

For this purpose a comparative study was made of the concentration of the body fluid of *Lycastis indica* acclimated experimentally in the laboratory to a variety of salinity conditions. The concentration of body fluid was estimated in terms of the chloride content since chloride forms the major component of the total ionic components of the animal. To obviate the difficulty in collection of sufficient quantity of body fluid, extract of the entire animal was used for estimation of the chloride content. Such procedure may be justified in view of the observation of Smith (1955) in *N. diversicolor* that values of chloride in extracts of the entire animal accord with those of the coelomic fluid.

Material and Methods

Experimental animals were acclimated to sea water of salinity ranging from 6·3‰ to full strength. Shock effects, that may result from sudden transfer of animals from normal medium to one more diluted, were avoided by stepwise dilution (Wells and Ledingham 1940).

For the measurement of chloride content, Conway's microdiffusion method was employed (Conway 1950). The method involves the oxidation of chloride to chlorine by a suitable acid permanganate mixture in the outer chamber of the 'Standard Unit'. The chlorine so formed is absorbed by potassium iodide in the central chamber of the unit, where it liberates an equivalent amount of iodine. The amount of iodine is estimated by titrating it against sodium thiosulphate.

The worms used for experiments were weighed correct to a milligram, and ground to a paste with a volume of glass distilled water according to the procedure followed by Conway (1950). After adding 0·2 ml of 0·0667 *N* sulphuric acid and 0·2 ml of 10% sodium tungstate, the mixture was centrifuged. The supernatant was separated and 2 ml sample was transferred to the outer chamber of the Conway unit together with 0·5 ml of 60% sulphuric acid and 0·2 mg of potassium permanganate. 1 ml of 20% potassium iodide was introduced into the inner chamber of the unit. After

two hours the contents of the inner chamber were titrated against 0·05 *N* sodium thiosulphate from the horizontal Conway microburette using 0·2% starch as indicator.

During each estimation, in one of the units, instead of the sample, 1 ml of 0·0141 *N* hydrochloric acid was introduced into the outer chamber and the procedure described earlier was repeated to find out the chlorine equivalent of 0·05 *N* sodium thiosulphate. Chloride present in 100 mg of tissue was calculated and the results expressed in grammes per 100 g of tissue. Wet weights of tissue were used in the calculation as it has been reported that tissues on drying lose as much as 31% of the original chloride content (Sunderman and Williams 1931).

For estimation of chloride in the external media of the experimental animals, 0·1 ml of the test sample was diluted to 10 ml and 1 ml of such diluted sample was introduced into the outer chamber of the Conway unit. The rest of the procedure was identical to that applied for the estimation of chloride in the animal extract referred to above. Using the chloride equivalent, the content of 0·01 ml of the sample used for titration was calculated and the results expressed in grammes per 100 ml of the external medium.

Chloride and salinity determinations of the test samples of water were also made, using silver nitrate titration method (Smith 1955). The chloride values estimated as above and expressed in terms of grammes per 100 g do not differ appreciably from the chloride values obtained by Conway's micro-diffusion method expressed in grammes per 100 ml of the medium. It has been suggested by Robertson (1949) that, for the comparison of equilibria, analysis should be in parts per unit weight of water or molality and not on volume basis, which does not take into account the space occupied by the colloids. Hence, in the present study, the total weight of the animal is taken into consideration and the chloride values of the animal expressed in grammes per 100 g. The results are shown in Tables I, II and Fig. 1. Each of the values for chloride is based on averages of 4 to 9 determinations.

Results and Discussion

From a comparative study of *N. diversicolor* and *Perinereis cultrifera*, Beadle (1931, 1937) observed that, in low dilutions of sea water, the former showed less increase in weight than the latter. Further, the weight remained constant at the maximum level in *Perinereis cultrifera* while in *N. diversicolor* the weight tended to decrease after an initial rise. The euryhalinity of *N. diversicolor* is attributable to its ability to resist and regulate the inflow of water better than the stenohaline *Perinereis cultrifera*. *Lycastis indica* is also able to tolerate wide variations in the salinity of the external medium. In a previous study it has been shown that, like the euryhaline *N. diversicolor*, it is able to regulate weight changes consequent on inflow of water due to

dilution of the external medium (Mary 1967). It was observed that experimental animals acclimated to sea water of salinity 34°/$_{oo}$ for about 30

TABLE I

Range of chloride concentration of the body fluids of L. indica *in relation to that of the media*

External medium			Internal medium
Sea water in percentage (%)	Salinity in parts per thousand (°/$_{oo}$)	Chloride of the external medium by Conway's method in g/100 ml	Chloride of the body fluid when in equilibrium with that of the external medium in g/100 g
100	36·910	2·225	0·950
70	26·240	1·952	0·577
50	19·043	1·363	0·475
30	11·970	0·775	0·357
20	9·281	0·527	0·291
12	6·287	0·347	0·258
2	0·870	0·048	No survivors

TABLE II

Degree of hypotonicity of chloride

Chloride content of the medium estimated by $AgNo_3$ titration method in g/100 g	Chloride content of the animal estimated by Conway's method in g/100 g	Difference between the chloride content of the animal and that of the medium in g/100 g	Percentage deviation from the isotonic condition (%)
2·039	0·950	1·089	53·42
1·450	0·577	0·873	60·20
1·052	0·475	0·577	54·86
0·661	0·357	0·304	45·99
0·513	0·291	0·222	43·08
0·347	0·258	0·089	25·65

days and subsequently subjected to osmotic stress by transference to dilute sea water of salinity 6°/$_{oo}$ showed a maximum increase in weight of about 79% of its original weight within three hours of immersion and regained its original weight at the end of about 293 hours. Similarly worms acclimated to varying degrees of salinity showed different degrees of weight regulatory ability under the same osmotic stress. There is a tendency to regain the

original weight whatever be the initial increase in weight on dilution of the external medium. The mechanism of weight regulation in polychaetes appears to vary. In *Lycastis indica*, it is noted in the present investigation that the worms with an internal chloride concentration of 0·58 g/100 g showed a maximum increase in weight amounting to 41% of the original

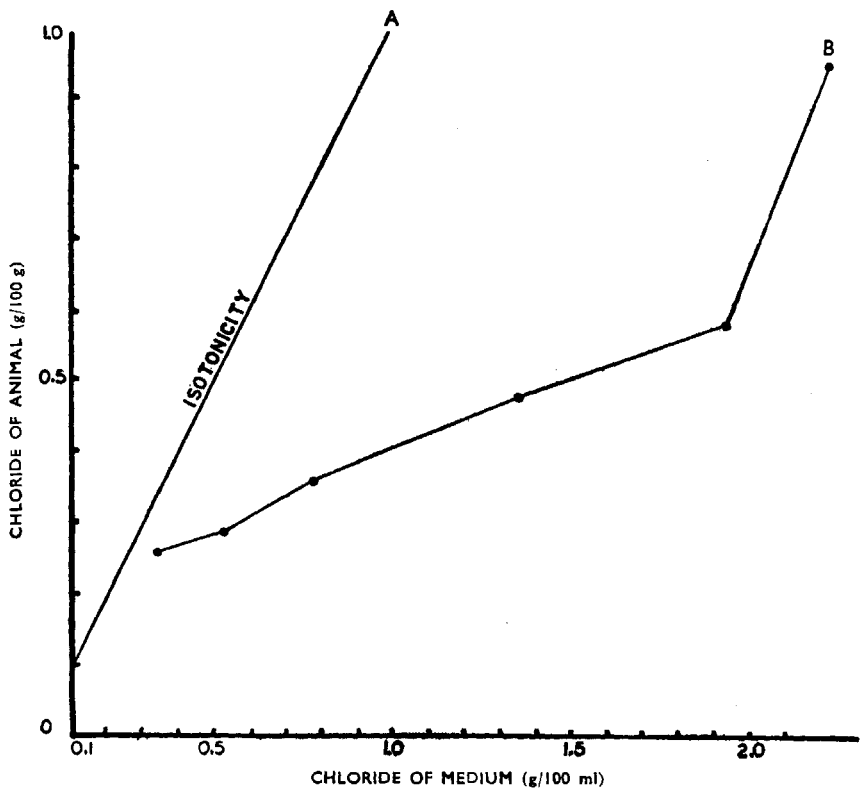

FIG. 1. Showing the changes in the chloride content of *Lycastis indica* in relation to that of the experimental medium.

Curve A— Isotonic condition, assuming the chloride concentration of the animal and that of the experimental medium are equal.

Curve B— Chloride concentration of the experimental animals in relation to that of the experimental medium.

Abscissae—Chloride content of the experimental medium expressed in g/100 ml.

Ordinate— Chloride content of *Lycastis indica* expressed in g/100 g.

when transferred to dilute sea water of chloride concentration of 0·35 g/100 ml, while the worms with internal chloride value of 0·36 g/100 g, under the same osmotic stress, showed much less increase in weight which was only 4% of the original weight. It may be inferred that the increase in weight is dependent on the salinity gradient. If the internal concentration is

less, the initial increase in weight is correspondingly less and hence such forms show a better weight regulatory ability.

The internal chloride concentration of *Lycastis indica* shows a correlation with that of the external medium. For a range of 0·26 g/100 g–0·95 g/100 g of internal chloride concentration, the corresponding value of the chloride concentration of the medium is 0·35 g/100 ml–2·23 g/100 ml (Table I). It is seen that the internal chloride content is maintained at a level lower than that of the external medium (Table II and Fig. 1). In view of the correlation seen between the internal concentration and initial increase in weight referred to above, it may be suggested that the maintenance of chloride content below that of the external medium is an adaptation to prevent osmotic swelling when subjected to changes in the salinity of the external medium. Smith (1963) reported that, in the nereids studied by him, the rate of salt loss decreased in proportion to the degree of lowering of salinity tolerated by them in their natural habitat. He suggested that the ability to lose salt 'might be considered as a favourable adaptation in a polychaete subjected to a sudden lowering of environmental salinity, since by lowering the osmotic gradient the animal would be faced with less severe problem of volume control and of disposing of excess of water taken in osmotically'. The results reported in the present study indicate that, in sea water of chloride concentration 2·23 g/100 ml, *Lycastis indica* shows an internal chloride content of about 0·95 g/100 g. When transferred to media of lower chloride content, the internal chloride values showed a corresponding decrease. It may appear that *L. indica* loses chloride when subjected to low salinity conditions and such ability may be of effective survival value.

It is not clear how the animal loses salts from the body fluids. In the light of the observations of Smith (1963), it is possible that such loss may be brought about through increased discharge of urine through the nephridia which have been found to be markedly enlarged by comparison with other nereids (Krishnan 1952). Such loss may also take place through the general body surface and gut as noted in certain brackish water nereids.

Another feature noted in *Lycastis indica*, which is of importance in this context, is its ability to tolerate a wide range of internal chloride concentration ranging from 0·26 g/100 g to 0·95 g/100 g. Similarly such tolerance has been reported in *N. diversicolor* (Smith 1955), *Arenicola marina* (Schlieper 1929), *Gunda ulvae* (Weil and Pantin 1931) and *Mytilus californianus* (Fox 1941) which are also able to survive wide variations in salinity of the external medium. In *L. indica*, the tissues become acclimated to a wide range of chloride concentration and at a level below that of the medium. Hyporegulation of chloride has been reported in *Marphysa gravelyi* (Krishnamoorthy 1963) and in *N. diversicolor*, Smith (1955) noted a tendency for hypotonicity of chloride in media showing chloride concentration higher than 5 g/l. The

mechanism of hyporegulation of chloride in *Lycastis indica* may involve ability to excrete chloride as well as for taking up water.

Hyporegulation of chloride appears to be a common feature of decapods tending towards semi-aquatic life. In such there is seen a correlation between low blood chloride concentration and ability to thrive in terrestrial conditions (Flemister 1958). In *Artemia salina*, the ability to maintain hypotonicity of haemolymph enables it to survive in highly saline media (Croghan 1958). Although *Artemia salina* resembles *Lycastis indica* in this respect, it differs from it in possessing the ability to maintain a hypertonicity of the haemolymph in dilute media. But, as suggested by Beadle (1943), such ability of *Artemia salina* is limited and appears to be a 'vestigial character'. In *Lycastis indica*, hyporegulation of chloride is an indication of its peculiar ability to tolerate a wide range of salinity conditions as well as its ability to lead a semi-aquatic life, for this species is able to survive exposure to air and was successfully made to live in moist blotting paper in the laboratory for several days. The results obtained from experimental studies on *Lycastis indica* support the suggestion made by Flemister and Flemister (1951) that animals which move from sea to brackish water possess the mechanism fundamental also to those that move from sea to land.

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* Not seen in original.