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STUDIES ON THE RELATIVE PERMEABILITY OF THE DIFFERENT REGIONS OF FEW SELECTED CRUSTACEANS*

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ABSTRACT

Leschkes' test was performed on the cuticle samples of *Ocypode platytarsis, Metapenaeus monoceros, Emerita asiatica* and *Ligia exotica*. Gill cuticle appeared to be markedly permeable to ions. The cuticle lining the gut in *Ocypode platytarsis* and *Metapenaeus monoceros* was also permeable, but to a lesser extent than the gill cuticle. The body cuticle of *Ocypode platytarsis* and *Metapenaeus monoceros* do not show permeability to Chloride. Permeability of the cuticle samples to water was tested by the dye penetration technique. It was noted that gill cuticle was markedly permeable. Next in degree of permeability was the cuticle lining the gut in *Ocypode, Metapenaeus* and *Emerita*. The body cuticle in all the three animals namely *Ocypode, Emerita* and *Ligia* was impermeable to dyes. In *Metapenaeus* the intermoult body cuticle showed permeability to water.

INTRODUCTION

FROM a comparative study of *Carcinus maenas, Homarus vulgaris, Maia squinado* and *Eriocheir sinensis*, Webb (1940) reported that specialised regions of the cuticle such as that covering the gills show increased permeability to water and ions. In this context it is relevant to recall the observations of Panikkar (1941) who noted that dyes like Methylene blue and neutral red penetrate more quickly the cuticle covering the gill and stain the gill lamella in *Crangon* and *Carcinus* while in *Leander* and *Palaeomonetes* the penetration of such dyes is very slow or absent.

It is suggestive that the euryhalinity of *Carcinus* may be attributed to the easy penetration of water through the cuticle covering the gills.

The differential permeability, to salts, of the cuticle covering the gills in crustaceans belonging to the species *Potamon sidneyi* and *Potamon depressus* reported by Ewer and Hattingh (1952) are passed on the observation that on treatment with silver salts, the four anterior gills absorbed and reduced the silver salts, while the three posterior gills did not show this property.

It has been suggested that the anterior gills are capable of absorbing chlorides in the natural environment of the animals while the posterior gills were probably not permeable to chlorides. A similar permeability of the cuticle covering the gills in *Artemia salina*, a hypo-regulator has been reported by Croghan (1958). The application of silver technique showed that the silver ions diffused into the cuticle of the gut and the first ten pairs of branchide showing thereby that they are permeable to salts. When the rest of the body cuticle was impermeable. But the cuticular features responsible for the permeability to salts in the gill regions are not known.

Previous work on water and ion control in *Ocypode* crab indicates that the principal organ concerned are the gills and the kidneys (Flemister and Flemister, 1951; Flemister, 1959). The regulation of water and ion transport

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across membranes covering the rest of the body are scarce. So it is felt that a survey of the cuticular permeability to salts and water at different regions may throw some light on the efficiency of osmo-regulatory mechanism of animals chosen.

Material and Methods

The crustaceans chosen for the present study are *Ocypode platytarsis, Emerita asiatica, Metapenaeus monoceros* and *Ligia exotica.*

Evidence of absorption of chloride ions has been provided by the application of Leschkes method (Copeland, 1948). The method consists of treatment of the test material with 10% silver nitrate made acidic with nitric acid.

The accumulation of reduced silver has been considered as evidence of localization of chloride. This depends on the formation of silver chloride on treatment with acid silver nitrate and subsequent reduction of silver by quinone containing solution (Mac Callum, 1905). A positive reaction with the technique is indicative of permeability of the cuticle to ions.

The materials used for the Leschkes test were the normal body cuticle in intermoult stages, the gill epithelium, foregut cuticle and the pericardial sac wall of the crustaceans. They were fixed without washing in 1% silver nitrate made acidic with nitric acid. The fixation was made in the dark.

The tissues were dehydrated embedded in paraffin and sectioned. The silver deposition that may result in the presence of chloride was located. The results obtained have been recorded in Table 1.

Permeability to water was also tested by what has been called the dye penetration technique. When the test animal was subjected to a dilute solution of methylene blue or neutral red the permeable region showed colouration and the impermeable region remained colourless. The results obtained have been recorded in Table 2.

Results and Discussion

Panikkar (1941) used a solution of Methylene blue and neutral red in sea water noted that in *Carcinus* and *Crangon*, at the end of half an hour the gills were coloured blue in contrast to *Leander* in which the gills failed to absorb the dye. From such observations it may be inferred that the cuticle covering the gills in *Carcinus* and *Crangon* are more permeable than that of *Leander.*

Ocypode platytarsis

When the test animal was subjected to the dye solution, no sign of dye could be detected in the body cavity suggesting that the normal body cuticle is impermeable to water. On the other hand the foregut cuticle was slightly coloured suggesting that the animal has the water drinking habit as a part of regulatory mechanism and the gut cuticle plays a role in absorbing water along with the salts when it is in dilute media and excreting the excess of water.

The pericardial sac and the gills were intensely coloured which could be detected even with the unaided eye. The intensity of colour developed appeared to be greater in the gills than in the pericardial sac. The development of colour appears to be dependent on factors such as the thickness and nature of the cuticle covering the regions concerned, the surface area exposed the extent and nature of the cavity enclosed and the diffusibility of the dye used.

With the Leschkes' test, granule were found in the pericardial sac, but less in intensity com-

pared to those found in the gill and were found arranged in a line roughly below the cuticular wall of the pericardial sac suggesting thereby that both are permeable to salts, but to different degrees.

was stained deeply with Methylene blue. The foregut cuticle when separated from the animal was found to take up stain, but in the intact condition the foregut was not stained. This probably suggests that these animals

TABLE 1. *Results of the silver staining technique obtained with the cuticles from selected regions of the animals*

Species	Region tested	Result	Localisation
Ocypode platytarsis	Normal cuticle	+	Black granules are found below the endocuticle and above the epidermis.
	Gills	+++	Black granules occur in the cytoplasm of the epithelium.
	Pericardial sac	+	Black granules occur below the cuticle.
	Fore gut wall	—	No evidence of black granules.
Metapenaeus monoceros	Normal cuticle	+	In the cuticular matrix.
	Gills	++	Cytoplasm of the epithelium.
	Fore gut	+	Gut wall.
Emerita asiatica	Normal cuticle	—	No granules found.
	Gills	++	Black granules occur below the cuticle.
	Fore gut	—	No granules occur.
Ligia exotica	Normal cuticle	—	—
	Pleopod cuticle	+	Black granules occur in the cuticle.

+ : Positive +++ : Intensely positive — : Negative

Metapenaeus monoceros

In *Metapenaeus* the body cuticle and the gills were stained black. The foregut cuticle also showed a similar condition suggesting that it is permeable to chlorides. The body cuticle was stained feebly and the gill cuticle

do not possess the water drinking habit as a part of osmoregulatory mechanism eventhough the cuticle lining it may be permeable to water.

Emerita asiatica

Emerita is a non-regulator and does not

stand prolonged washing with distilled water for removing the chlorides adhering to their body surface and so the duration of washing was reduced to few minutes. There was no evidence of chloride below the body cuticle.

TABLE 2. *Results of the dye penetration technique obtained with the cuticle from selected regions of the animals*

Species	Name of the organ	Result
Ocypode platytarsis	Body cuticle	—
	Gill epithelium	+++
	Fore gut epithelium	++
	Pericardial sac	++
Metapenaeus monoceros	Normal cuticle	++
	Gills	+++
	Fore gut cuticle	+
Emerita asiatica	Normal cuticle (Intermoult)	—
	Gill	++
	Fore gut cuticle	+
	Normal cuticle (Post moult)	++
Ligia exotica	Normal cuticle	—
	Pleopod cuticle	+

+ : Positive; +++ : Intensely positive and — : Negative

It is suggestive that even where the cuticle is permeable to chloride it may not be retained, but pass through it. There was no evidence of black colouration in the foregut epithelium or the cuticle suggesting their impermeability to chloride.

The dyes were found to penetrate the fresh moult and post moult stage - cuticle. The gills were found to be stained intensely. The foregut cuticle was not stained in vivo although the isolated cuticle was found to be permeable to the dye solution. It may be suggested that the living animal avoids dilution of its body fluids by preventing inflow of water through the moult as a part of a regulatory device.

Ligia exotica

In *Ligia exotica,* the impermeable body cuticle is unaffected by the treatment with the dye solution, but the pleopod cuticle showed evidence of penetration of the dye to some extent.

It is evident therefore, that, the species of Crustacea studied, do not respond to the tests uniformly. Several factors such as the texture, chemistry of the cuticle, the thickness of the cuticle and the physical condition may be ascribed to this.

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