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ECOPHYSIOLOGY OF SALT ACCLIMATISATION IN CRUSTACEANS : A MINI REVIEW (*)

by

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SUMMARY

With their marine, fresh water and euryhaline representatives, crustaceans exhibit almost any of the known possible patterns of osmo-ionoregulation. That group therefore appears as a choice material to tackle the question of ecophysiology of salt acclimatisation from a comparative point of view. In crustaceans, osmo-ionoregulation can be effected in two different ways the significance of which is always to avoid water movements at the cellular level. The first one, of general occurrence and considered as a prerequisite for adaptation to salinity changes, is to maintain the intracellular fluid isosmotic to the extracellular fluid, either the body fluids, or that of the environment. The second one is to control the concentration of the extracellular fluids at a more or less constant level regardless of the external salinity. This review will focus on the second way where mechanisms are active essentially in boundary epithelia. The gills will be shown to be the prominent structure responsible for the blood NaCl balance and regulation in marine, marine euryhaline and brackish water species. The review will therefore deal mostly with recent physiological and ultrastructural data on gill tissue and provide information leading to a characterization of the particular mechanisms and driving forces at work at that level. It will refer largely to experiments using perfused preparations of gills isolated from the chinese crab *Eriocheir sinensis,* taken as a model. The applicability of the chinese crab model to other crustaceans will be considered. It will be shown also that the cuticle lining the epithelium is largely involved in ionic regulation in crustaceans. It does contribute indeed to reduce ionic leaks in regulators and yet allows for the entry of ions across specific « channels » at the sites where active uptake takes place. An attempt is made to understand how both the cuticle and the epithelium fit in a working epithelio-cuticular complex.

Keywords : Crustacea, cuticle, ecophysiology, epithelium, gill, ionoregulation, Na^+, Cl^-, osmoregulation, potential difference, ultrastructure.

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INTRODUCTION

A review centered on the Ecophysiology of salt acclimatisation in crustaceans should consider all the processes at work in the control of the thermodynamic activity of water in the different biological fluids of the species considered. In crustaceans, the inorganic ions Na^+ and Cl^- are the major osmotic effectors found in these fluids, either extracellularly or intracellularly, while in addition cellular fluid contains significant amounts of amino acids and peptides of actual osmotic importance. Such a review should therefore consider any kind of metabolic and transport process involved in the adjustment of the level of both these inorganic and organic constituents. Viewed in this way, the subject is very complex and much too voluminous to be dealt with in such a brief review. As a matter of fact, for many years, the composition of the haemolymph has been studied in the various groups of crustaceans as a function of the salinity of the environment. All together, these investigations represent a huge amount of information which corroborates the

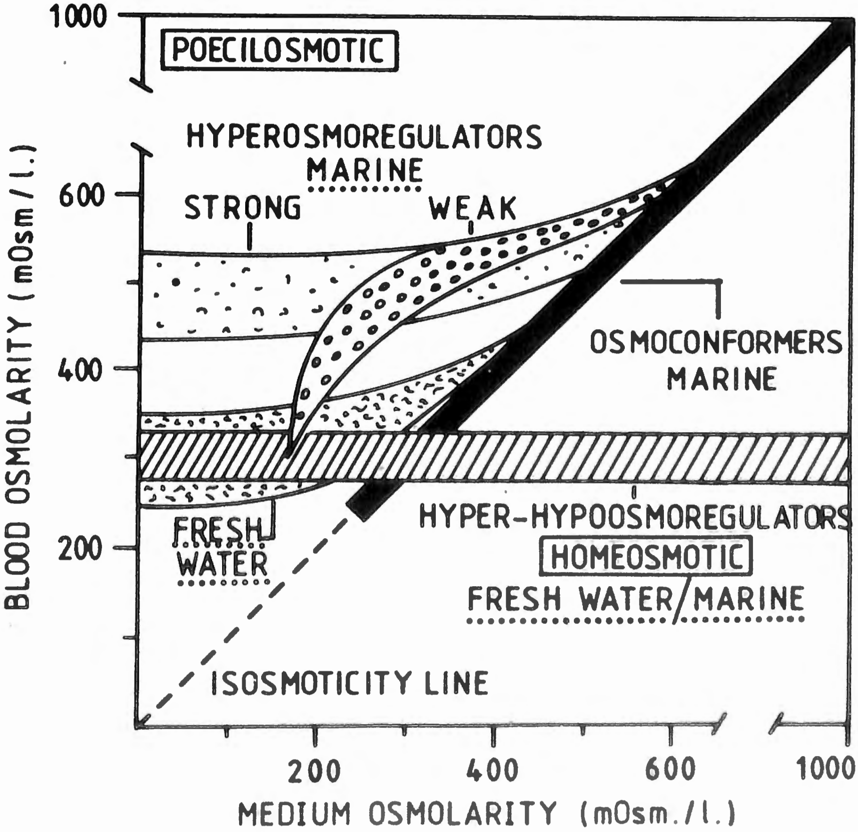

Fig. 1. — Patterns of haemolymph osmoregulation in aquatic crustaceans

assertion that crustaceans exhibit almost all of the possible patterns of haemolymph composition. From that abundant material, it has been possible to draw several generalizations for the most representative patterns encountered in animals living in a range of media from concentrated sea water (SW) to freshwater (FW) and terrestrial environments (Fig. 1).

A first and basic way to effect osmotic regulation is to maintain the intracellular fluid isosmotic to the extracellular fluid, either of the external medium or of the body fluid. In media of fluctuating salinities, the demand of the medium may then be difficult to meet. All the mechanisms involved in this kind of regulation or adjustment have been gathered under the general heading « Isosmotic regulation of cellular fluids ».

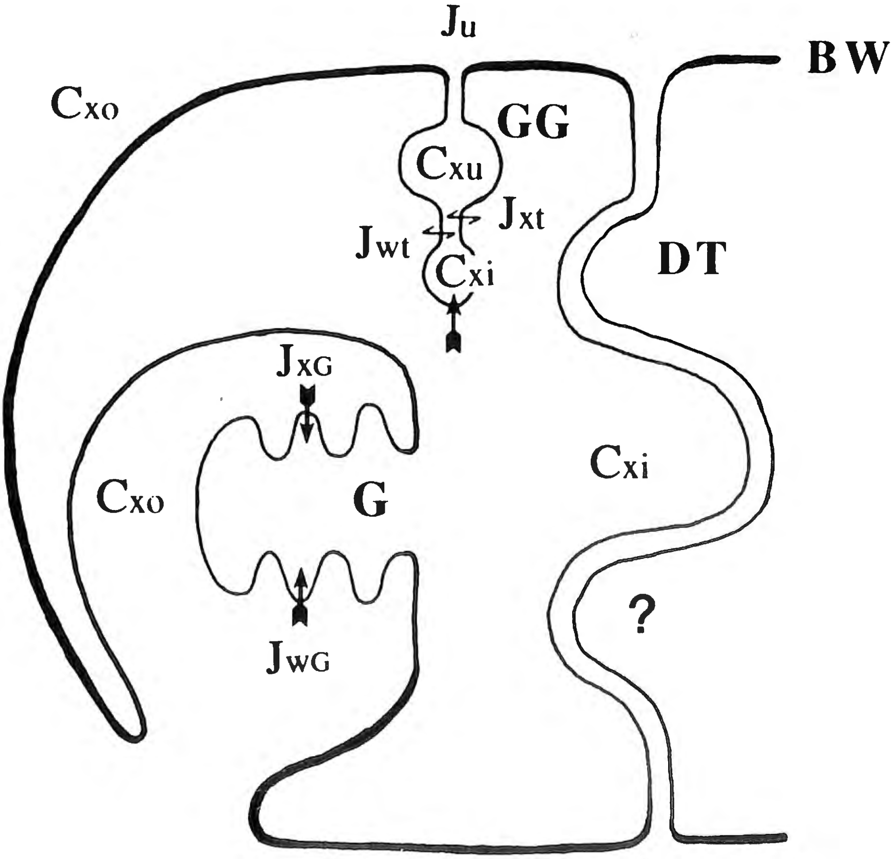

Fig. 2. — Schematic representation of a theoretical crustacean.
BW : body wall ; C : concentration ; DT : digestive tract ; f : filtrate ; G : gill ; GG : green gland ; i : internal medium (haemolymph) ; J : flux ; o : outside (external medium) ; t : tubule ; u : urine ; w : water ; x : inorganic compound x.

Another process to achieve osmoregulation has been evolved by many species, a process working always aside from the mechanisms of isosmotic regulation of intracellular fluid. That second way is to maintain the osmotic concentration of the extracellular (body) fluids more or less constant regardless of the salinity of the surrounding medium. These processes ensure an « Anisosmotic regulation of the body fluids ». They preserve internal tissues from drastic and sudden salinity changes that could impair life mechanisms of the cell, or, at least, induce costly adjustments. Both processes work together to control water movements at the cellular level.

This review intends to concentrate on this latter category of crustaceans, *i.e.* on species that hyperregulate in dilute media and that are osmoconforming in salt concentrated ones. Such animals do achieve anisosmotic regulation of their blood in dilute media.

Two types of basic mechanisms relevant to the « pumps and leaks » system are implicated in the control of the blood NaCl level : (1) « limiting processes » acting on the permeability properties of epithelial structures in order to minimize the diffusive movements of ions ; (2) « compensatory processes » driving active movements of NaCl to counterbalance the diffusive fluxes. These mechanisms appear to be at work essentially in boundary epithelia, such as the body walls, the gut, the excretory organs, and the gills (Fig. 2). Their study has been reviewed regularly and the reader interested in an overall panorama of this literature since the early 1960's is referred to the following review articles and volumes : POTTS and PARRY, 1964 ; SCHOFFENIELS and GILLES, 1970 ; GILLES, 1975 ; LOCKWOOD, 1977 ; GREENAWAY, 1979 ; SPAARGAREN, 1979, KIRSCHNER, 1979 ; GILLES, 1979 ; MANTEL and FARMER, 1983 ; GILLES and PÉQUEUX, 1983, 1985 ; PÉQUEUX and GILLES, 1988. Compensatory influx of NaCl in the gut appears to be related mainly to water absorption in hypo-osmoregulators ; the role of this organ in the overall blood salt balance and osmoregulation is, however, far from being clear. Compensatory NaCl reabsorption has also been described in the excretory organs of some freshwater species. Such a mechanism has never been found in brackish water species nor in marine ones. Clearly, the gill appears up to now as the prominent structure implicated in blood NaCl balance and osmoregulation.

STRUCTURE AND FUNCTION OF THE CRUSTACEAN GILL EPITHELIUM AS RELATED TO ENVIRONMENTAL SALINITY

Since the early works of KROGH (1938, 1939) active uptake of NaCl at the gill level has been considered to play an essential part in blood osmoregulation in many hyper-regulating aquatic species. Since then, indirect evidence that supports the model has been accumulating while most of these experiments were performed on whole animals. This failed to provide conclusive information leading to a clear characterization of the particular mechanisms and driving forces at work at the gill level. Part of this problem has been overcome during the past years with the use of perfused preparations of isolated gills. It is clear that much of our understanding of the physiology of that organ has been gained in investigations on such preparations. The biochemical approach which has been developed concurrently will not be considered here. It deals essentially with the two enzymes Na^+, K^+ ATPase, and carbonic anhydrase. An account of the function of carbonic anhydrase in crustacean gills has been given by HENRY (1984) and BURNETT (1984). The reader interested in Na^+, K^+ ATPase is referred to different papers on the topic : TOWLE *et al.*, 1976 ; PÉQUEUX and GILLES, 1977 ; SPENCER *et al.*, 1979 ; NEUFELD *et al.*, 1980 ; PÉQUEUX and CHAPELLE, 1982 ; SIEBERS *et al.*, 1982 ; PÉQUEUX *et al.*, 1983, 1984 ; PÉQUEUX and GILLES, 1984, 1988 ; TOWLE, 1981, 1984a,b.

The results obtained following this line of approach, *i.e.* experimentation on isolated perfused gills, clearly establish that a distinction, or even in some species like the chinese crab *Eriocheir sinensis* (H. Milne-Edwards) a clear-cut distinction, must be made between the three most posteriorly located pairs of gills and the anterior ones. Several sets of evidence suggest indeed that the various pairs of gills of decapods are not functionally equivalent. This has been indicated by experiments on histology and ultra structure (COPELAND and FITZJARRELL, 1968 ; BARRA *et al.*, 1983), as well as on characterization of ions fluxes (KOCH *et al.*, 1954 ; PÉQUEUX and GILLES, 1981).

As a matter of fact, if all gills of crabs are macroscopically similar, microscopic and ultrastructural observations indicate they are lined by quite different epithelia. The epithelium of the anterior gills is much thinner (2-4 µm) than in the posterior ones (10 µm and more). There is little or no folding of the plasma membrane and the extracellular space under the cuticle is extremely reduced. The amount of intracellular organelles seems also to be very limited (BARRA *et al.*, 1983). Clearly, these gills appear to be lined only by an epithelium of the « respiratory » type. Conversely, the large epithelial cells of the posterior gills are characterized by a complex and well-developed network of apical folds which delimits a large extracellular compartment under the cuticle when animals are acclimatised to dilute media. Mitochondria are the most abundant organelles in these cells ; they may fill almost completely the cytoplasmic space. Basolaterally, another important infolding system coming into tight contact with mitochondria penetrates deeply into the cells (BARRA *et al.*, 1983). This kind of structure appears to be quite characteristic of « salt-transporting » epithelia, it is found indeed in most tissues implicated in ion transport and osmoregulation (BERRIDGE and OSCHMAN, 1972 ; TAYLOR and TAYLOR, 1992).

As stated above, these differences in structural organization of gills of euryhaline crabs when in dilute media have functional correlates. Early experiments of KOCH (1934) studying the staining of gills with silver salts already suggested about 50 years ago, that gills pairs showing fixation of silver, *i.e.* the three posterior pairs, are the only ones involved in active Na^+ uptake. This has been largely confirmed later on by experiments using perfused preparations of gills and radioisotopes. From the data reported in Table 1, it can be concluded indeed that the epithelium of the anterior gills is permeable to Na^+ ions but quite impermeable to Cl^-. It has been established that Na^+ movements are essentially passive and fit in with the

TABLE 1

NaCl flux in perfused Gills isolated from the crab *Eriocheir sinensis*.

Acclimation medium	Na^+ or Cl^- in saline (mM)			ANTERIOR GILLS		POSTERIOR GILLS	
	OUT		IN	Influx (µequiv./gww/h)	Efflux	Influx (µequiv./gww/h)	Efflux
Na^+ flux							
FW	240	Na^+	240	285 ± 87	277 ± 84	260 ± 61	N.S.
	1		240	6 ± 2	112 ± 74	21 ± 7	N.S.
	10		240	85 ± 14	247 ± 13	142 ± 60	N.S.
SW	500	Na^+	480	3448 ± 960	2079 ± 632	N.S.	N.S.
Cl^- flux							
FW	280	Cl^-	280	N.S.	N.S.	2273 ± 539	875 ± 168
	1		280	N.S.	N.S.	16 ± 3	138 ± 39
	10		280	N.S.	N.S.	568 ± 66	469 ± 2
SW	560	Cl^-	560	N.S.	N.S.	2353 ± 826	2514 ± 724

Mean results of at least 5 experiments ± S.D.
N.S.. = not significant ; FW = fresh water ; SW = sea water.

Ussing's equation of the fluxes ratio. Conversely, there is no Na^+ efflux in the posterior gills, while there is an influx that must therefore be linked to the activity of active processes. Cl^- ions cross the epithelium in both ways, but influx generally overcomes the efflux. It is clear that posterior gills only are able to transport NaCl transepithelially against a concentration gradient. Further studies conducted on these perfused preparations have uncovered a number of transport processes located in the apical and the basolateral membranes and that can yield a tentative cellular model accounting for the NaCl absorptive process observed when animals are acclimatised to dilute media (for review, see for instance PÉQUEUX and GILLES, 1988).

A MODEL OF NaCl TRANSPORT IN *E. SINENSIS* POSTERIOR GILL — APPLICABILITY TO OTHER CRUSTACEANS

A schematic drawing taking into consideration the different experimental results available at present is given in Fig. 3 . While this model has to be considered as basically tentative, it agrees with the data obtained when both sides of the preparation are bathed with the same saline i.e., in the absence of a transepithelial concentration gradient. It is worth noting that in the freshwater chinese crab, the

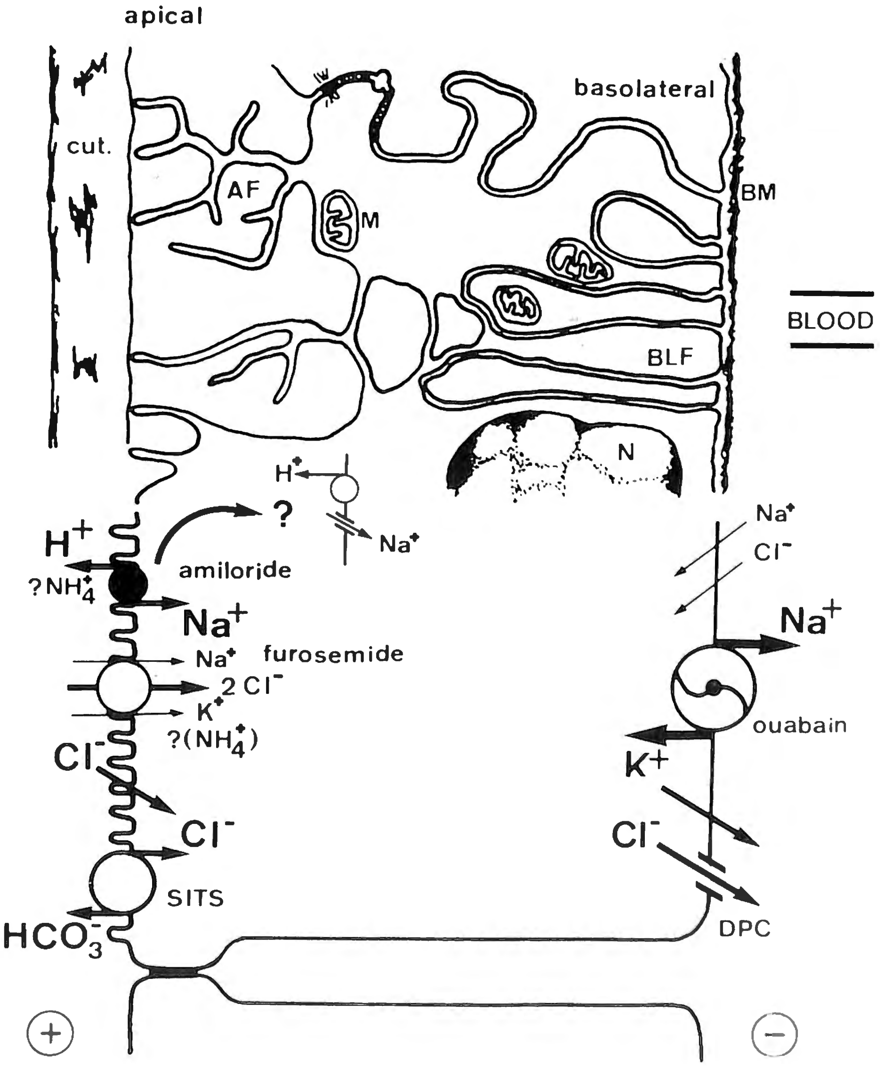

Fig. 3. — Functional model of POSTERIOR gills of FW chinese crabs, *E. sinensis*. Both sides of the epithelium are bathed with the same « FW bloodlike » saline. Explanations in the text.

intracellular level of Na^+ and Cl^- in the gill epithelium is higher than in the external medium and lower than in the serosal/haemolymph one. Therefore, the movements of these ions from the external medium to the intracellular and haemolymph compartments cannot occur by diffusion only. Active transport processes at both sides have to be taken into consideration. To be pointed out are the few following distinctive features : (1) Na^+ and Cl^- are transported independently ;

(2) the permeability of the epithelium to Na^+ is extremely low ; (3) the epithelium is quite permeable to Cl^- ; (4) the basic driving mechanism is a Na^+/K^+ pump whose activity is linked to a $(Na^+ + K^+)$ATPase system and which has been established to reside in the basolateral membranes ; (5) a NaK2Cl mechanism is thought to be at work at the apical side of the epithelium and involved in the entry of Na^+ and Cl^- into the cell ; (6) Na^+ inward movement at the apical side also seems to be largely related to an amiloride-sensitive Na^+/H^+ exchange process ; (7) a SITS-sensitive Cl^-/HCO_3^- exchange system is involved in the transport of Cl^- at the apical side ; (8) Cl^- ions leave the cell at the basolateral side using a Cl^- conductive pathway which is very likely driven by the K^+ concentration gradient known to generate a potential difference oriented haemolymph side positive.

The question which arises now is that of whether the *E. sinensis* model of gill transport can be applied to other crabs , as for example the shore crab *Carcinus maenas* (L.), a well-known weak regulator. This model is by no means exclusive of others. However, it could be quite satisfactorily applied to other euryhaline crabs by adding pathways for Na^+ outward movements or by considering that the transport characteristics of their gills are a mixture of the characteristics described separately for the anterior and posterior gills of the chinese crab.

The relative importance of these processes and/or of these specific characteristics could vary greatly from one species to another, thus determining their ability to hyperosmoregulate in dilute environments. In *C. maenas*, the anterior gills are mostly lined with a respiratory-like epithelium, while both a respiratory and a salt-transporting epithelium are found in the posterior gills. However, the salt-transporting tissue never exceeds 30 % of the whole lamellar surface (COMPÈRE *et al.*, 1985 ; 1989). The large unidirectional Na^+ fluxes that occur in *C. maenas* gills (WANSON and PÉQUEUX, 1981 and unpublished results), at variance with the situation found in *E. sinensis*, could thus be satisfactorily related to the fact that in *E. sinensis*, the posterior gills are essentially lined with an epithelium of the transporting type (with a low Na^+ permeability), while in *C. maenas*, both the respiratory (with a high Na^+ permeability) and the transporting (with low Na^+ permeability) epithelia are present. A similar situation would also occur in the blue crab *Callinectes sapidus* (Rathbun) (COPELAND and FITZJARREL, 1968).

These results lead to the interesting conclusion that the structural organization of the gills can be directly related to their functional properties, hence to the osmoregulatory capabilities of the considered species. They substantiate the idea that the model of gill structural and functional organization described for the chinese crab *E. sinensis* can be applied quite satisfactorily to other euryhaline crabs. They moreover establish that this species is an almost unique model with respect to the study of not only ion transport processes, but also the structure-function relation in a NaCl-transporting epithelium. It is however not actually complete, and a precise view of the mechanisms at work in the gill tissue of crustacea, and also of the mechanisms involved in the overall osmoionoregulation function in crustacea entails the study of the physiological properties of a structure which always has been ignored by the physiologists : the cuticle.

PERMEABILITY PROPERTIES OF THE CRUSTACEAN CUTICLE

Up to now, most of the physiologists have taken for granted that the thick cuticle covering the body is impermeable while that of the gills is highly permeable, which implies that the permeability of crustacea only depends upon the gill epithelium.

Recent work however demonstrated that this is far from being the case and that the various cuticles may exhibit various degrees of permeability that can be related to the osmoregulatory performances of the species considered (for review, see LIGNON and PÉQUEUX, 1990 ; PÉQUEUX and LIGNON, 1991).

These studies have been conducted by measuring, in a comparative way, diffusional transcuticular potentials and electrical conductance in several species of decapod crustaceans. It is established that the cuticle permeability depends upon the species considered and its ionoregulation capability, upon the localization of the gill in the gill chamber or even upon the topographic region in a single gill and upon the nature of the ionic species.

In each case, the cuticle must always be considered as a diffusion barrier for the main osmotic effectors, Na^+ and Cl^-. However, the efficiency of this barrier is low in the case of the stenohaline osmoconformers. In hyperregulators, the efficiency of the cuticular barrier is much higher. What emerges from the studies of Lignon and coworkers is that a clear-cut correlation can be established between cuticular ionic permeabilities, either branchial either extrabranchial, and the environmental salinity that can withstand the three following groups : (1) marine conformers (*Homarus gammarus* L., *Maia squinado* Risso and *Nephrops norvegicus* L.), (2) moderate regulators (*Carcinus maenas*) and euryhaline conformers (*Cancer pagurus* L.) and (3) powerful regulators (*Astacus leptodactylus* Escholtz and *Eriocheir sinensis*). For instance, soft or calcified cuticles of the body and of the gut are 500 to 5,000 times less permeable in regulators as compared with conformers (LIGNON and PÉQUEUX, 1990). For each species, the gill cuticle is the most permeable while the carapace and the soft body cuticle are the least permeable. Anyway, the gill cuticle of strong regulators is less permeable than the carapace of osmoconformers.

To be pointed out also is the fact that, in hyperregulators, the cuticle exhibits important differences in its electrical charactéristics between the various pairs of gills or even between different topographic regions of the same gill. As a matter of fact, the cuticle permeability of the crayfish gill lamina is low for all ionic species but Cl^- while, in gill filaments and in crab gills, permeability to cations is high and permeability to Cl^- is low. In addition, the cuticle shows a functional asymmetry which favours ionic influxes, this asymmetry being almost inexistant in osmoconforming species.

It is thus clear that the cuticle behaves as a structure complementary to the epithelium with respect to ionoregulation. There is no doubt the reduced cuticular permeability of regulators when in dilute media results in the reduction of diffusional ionic leaks. It also forces cnsideration of the anatomical subcuticular space in the gill as a genuine physiological compartment where recycling of ions by the cellular uptake systems before they cross the cuticle could take place in an efficient way. Such a process could well account for the low « apparent » permeability of the epithelium reported above, for instance, in the posterior gills of the chinese crab in the case of Na^+ ions.

It is also remarkable that the body cuticle permeability is generally low enough foo most of the ionic exchanges take place at the gill level. However, this permeability is conversely large enough to account for a NaCl loss as large as the urine loss in the crayfish. Fron both these lines of evidence, the conventional view of an impermeable body cuticle and of a highly permeable gill cuticle in crustaceans appears actually as a very crude approximation.

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