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POSSIBILITIES OF OSMOTIC ADAPTATION OF THE SHRIMP *Palaemon adspersus* TO LOW SALINITY MEDIA

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ABSTRACT:

The effects of low salinities (8 and 5‰) on hydric and mineral balance of males and females of Palaemon adspersus were studied. In hypo-saline media, both sexes showed noticeable possibilities of osmoregulation due to modifications of passive permeability of the cell membranes and by stimulation of the active ionic transport mechanisms.

The present work is a part of the experimental series which we performed, concerning to the water and main ions (Na^+, K^+, Ca^{2+}) dynamics across the cell membranes of P.adspersus, in conditions of salinity decrease of the sea water.

It is known that the adaptation to the hypo-osmotics environments (as well as to the hyper-osmotics) involves the existence of an intermediary compartment - the extracellular fluid, having a buffer role between the external medium and living cells. That is why, any modification in the composition and osmolarity of the ambient medium assumes a direct intervention of the extracellular compartment in the regulation and control of the insit and exit of the water and salts across cell membrane with a

view to establishing and controlling the osmolarity of the intracellular fluid as an essential factor in maintaining the viability in the cells.

One of the usual methods employed in the research of the water distribution concerning the alternations in osmolarity of external medium consists in the measuring of extracellular space of tissues. This involves the calculation of the tissue water fraction in which a substance (e.g. inulin) to which the cell membrane is impermeable,is distributed in a similar concentration to that of the incubation medium. Thus, the intracellular water content can be deduced from the total tissue water content, after the correction for the extracellular water has been made. Simultaneously with the modifications of the hydric content,in the two compartments take place modifications of the ionic concentrations which implies a series of interactions between the solvent(water) and solute, solvent and membrane and the solute and membrane.

Some of these intricate and less known aspects, particularly regarding the Black Sea shrimps, in low salinity conditions, are presented in this paper.

MATERIALS AND METHODS

The experiments were performed on adult males and females (wanting in eggs) of Palaemon adspersus,in summer (July and September).The animals accomodated to normal sea water (17.5 g ‰ salinity) were transferred directly-without intermediary salinity steps to salinities of 8 ‰ and 5 ‰ respectively.The dilution of sea water was made with declorined tap water.

The measurements of the water, Na^+,K^+ and Ca^{2+} dynamics were performed to controls (C) and to intervals of 24, 48 and 96 hours from the moment of salinity decrease, on the two sexes, separately. Each experiment was performed on 5-6 animals, taking duplicates of tissue samples. One sample was dried to $105^{\circ}C$ for determining the total water content ($W_{tot.}$) as well as the total tissue content of the studied ions.The second sample was used for the measurement of the extracellular space, i.e. extracellular water content (W_e). The extracellular water was determined by using the inulinic space method, while the measurement of total

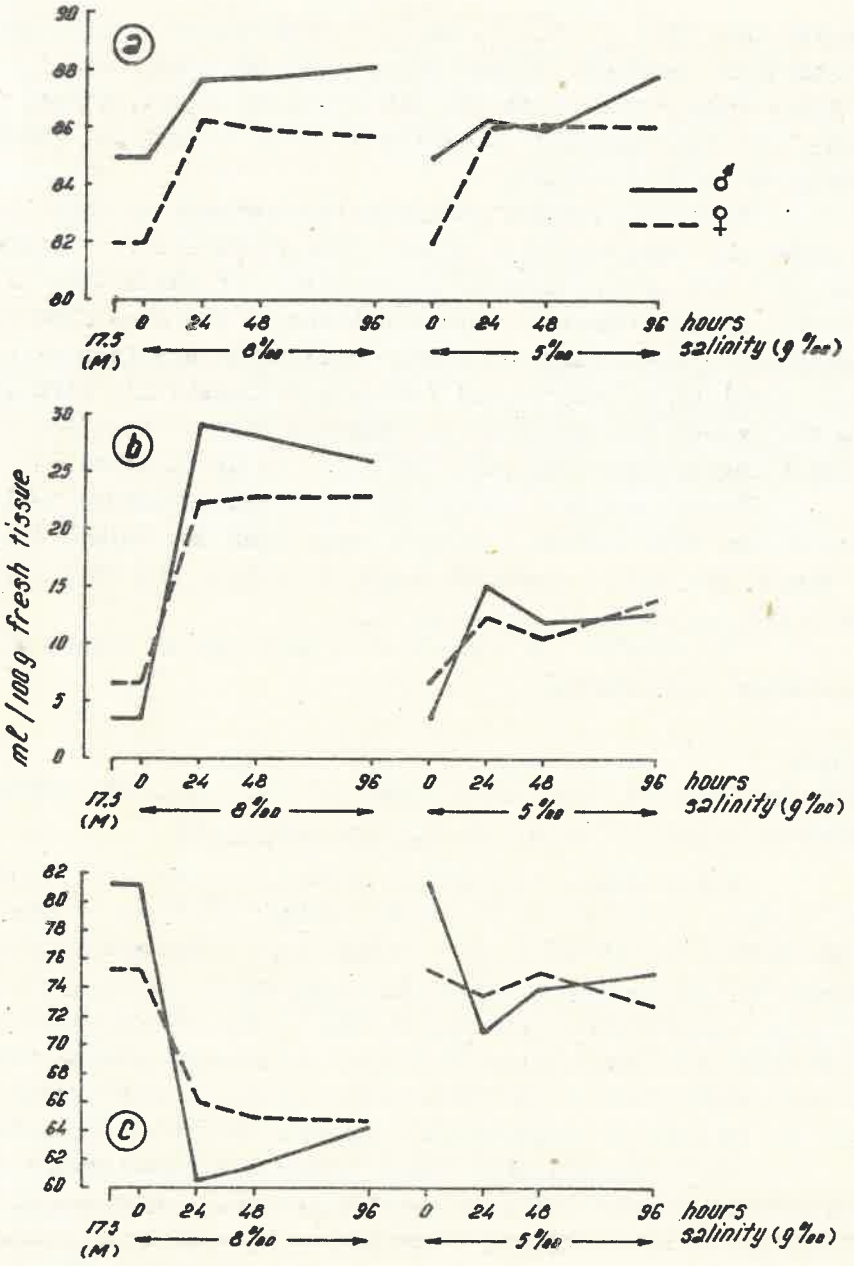

Fig. 1 - Water dynamics in the body tissues of the Palaemon adspersus shrimp after the decrease of the salinity from 17.5‰ to 8‰ and 5‰ respectively; a - total water; b - intracellular water; c - extracellular water

tissular ions ($Na^+_{tot.}$, $K^+_{tot.}$, $Ca^{2+}_{tot.}$) by flamephotometric method. Both parallel samples, before processing, were kept for 3 hours, for ionic equilibrium with the extracellular fluid, in sea water having the correspondent salinity of each moment in which the experiments were performed.

From the results obtained with respect to total tissular water and extracellular space, the intracellular volume of water (W_i) can be calculated. On the basis of these data as well as of the flamephotometric determinations of the ions from tissue samples and considering the ionic concentrations from extracell fluid $[Na^+_e]$, $[K^+_e]$, $[Ca^{2+}_e]$ equal (after equilibration) with those from the incubation solution, the content of ions from the intracellular compartment (Na^+_i, K^+_i, Ca^{2+}_i) can be calculated.

To get a clear picture on the dynamics across cell membrane of the ions studied, we have expressed the values obtained in terms of their concentrations in extra- and intracellular fluids (mg/100 ml W_e or mg/100 ml W_i).

The results are expressed as averages of values obtained from 5-6 individuals.

RESULTS

1.THE EFFECTS OF LOW SALINITIES OF SEA WATER ON HYDRIC AND MINERAL BALANCE TO MALES OF Palaemon adspersus

The decrease of external medium salinity from 17.5‰ to 8 ‰ caused, in the first 24 hours,a slight increase of the total water content accompanied by a significant enhancement of hydric content in extracellular space, and a decrease of that in intracellular compartment, simultaneously. In the following period up to 96 hours,the total water content is maintained almost constant at this high level as the reentrance of water into cells takes place on account of extracellular compartment(Table 1,Fig.1a,b,c).

Concomitantly with these hydric modifications, a series of important changes of ionic concentrations takes place, especially in the intracellular compartment. Thus,after 24 hours from the decrease of external medium salinity, the total Na^+ content of tissues is reduced to half because of the excessive diminution of its concentration into cells,below the extracellular level,and

which is maintained in these limits (with unimportant oscillations) throughout the experiment (Table 1, Fig. 2a,b).

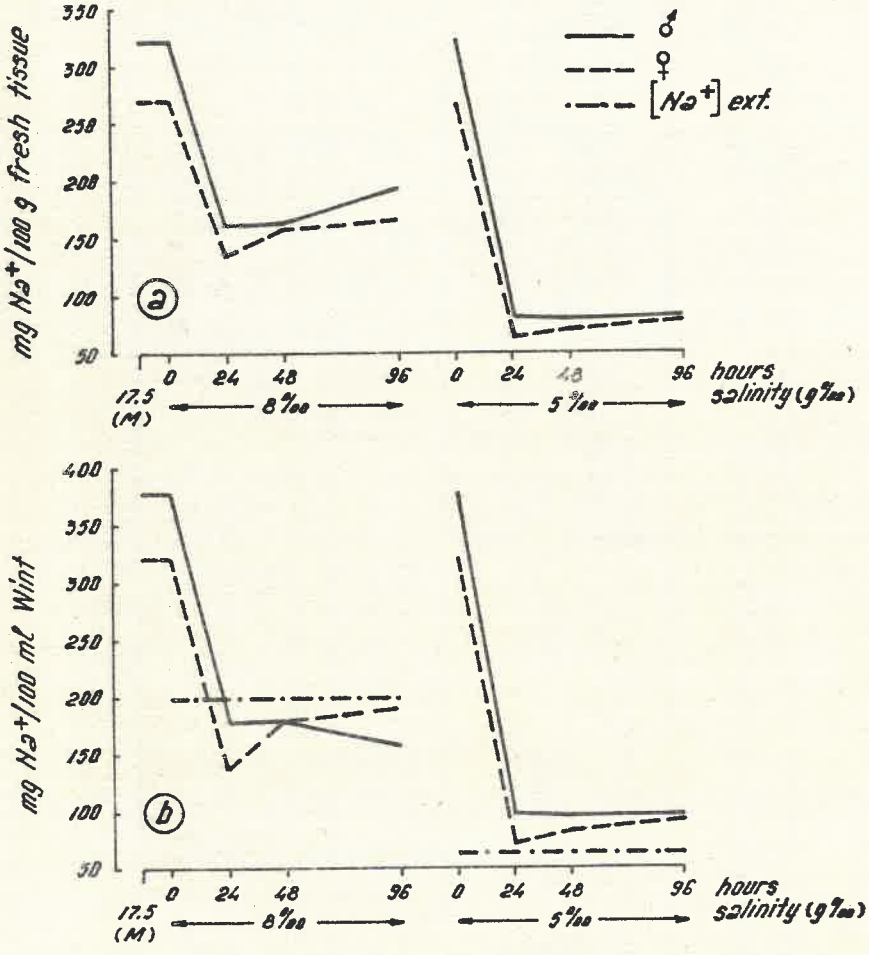

Fig.2 - Dynamics of Na^+ ion in the body tissues of the P.adspersus shrimp after the decrease of the salinity from 17.5‰ to 8 ‰ and 5 ‰ respectively; a - total tissue content; b - intracellular concentration

Table 1

The water and Na^+, K^+, Ca^{2+} dynamics across the cell membranes of the males of Palaemon adspersus in conditions of the low salinity of the sea water

Salinity (g‰)	Time (H)	Water				Natrium			Kalium			Calcium		
		Water total (ml/ loo g f.t.)	W_e (ml/ loo g f.t.)	W_i (ml/ loo g f.t.)	W_e/W_i	$Na^+_{tot.}$ (mg/ loo g f.t.)	Concentration $Na^+_{ext.}$ (mg/ loo ml W_e)	Concentration $Na^+_{int.}$ (mg/ loo ml W_i)	$K^+_{tot.}$ (mg/ loo g f.t.)	Concentration $K^+_{ext.}$ (mg/ looml W_e)	Concentration $K^+_{int.}$ (mg/ looml W_i)	$Ca^{2+}_{tot.}$ (mg/ loo g f.t.)	Concentration $Ca^{2+}_{ext.}$ (mg/ looml W_e)	Concentration $Ca^{2+}_{int.}$ (mg/ loo ml W_i)
17.5	o	84.89	3.53	81.36	o.o4	324.46	44o.oo	379.71	8o.o4	14.4o	97.75	2o.85	2o.oo	24.77
8.o	24	87.67	29.26	58.41	o.5o	162.6o	2o1.oo	177.69	33.38	1o.4o	51.94	17.66	9.5o	25.45
8.o	48	87.76	28.19	59.57	o.47	164.22	2o1.oo	18o.56	32.55	1o.4o	49.72	16.18	9.5o	22.66
8.o	96	88.o8	25.97	62.11	o.42	194.o4	2o1.oo	158.44	51.o4	1o.4o	77.83	12.35	9.5o	15.91
5.o	24	86.26	15.24	71.o2	o.21	8o.98	66.oo	99.86	47.73	5.5o	86.o2	21.18	9.25	27.84
5.o	48	85.91	11.91	74.oo	o.16	79.69	66.oo	97.o7	53.24	5.5o	71.o7	16.o3	9.25	2o.17
5.o	96	87.86	12.85	75.o1	o.17	81.38	66.oo	97.19	5o.58	5.5o	66.48	2o.31	9.25	25.49

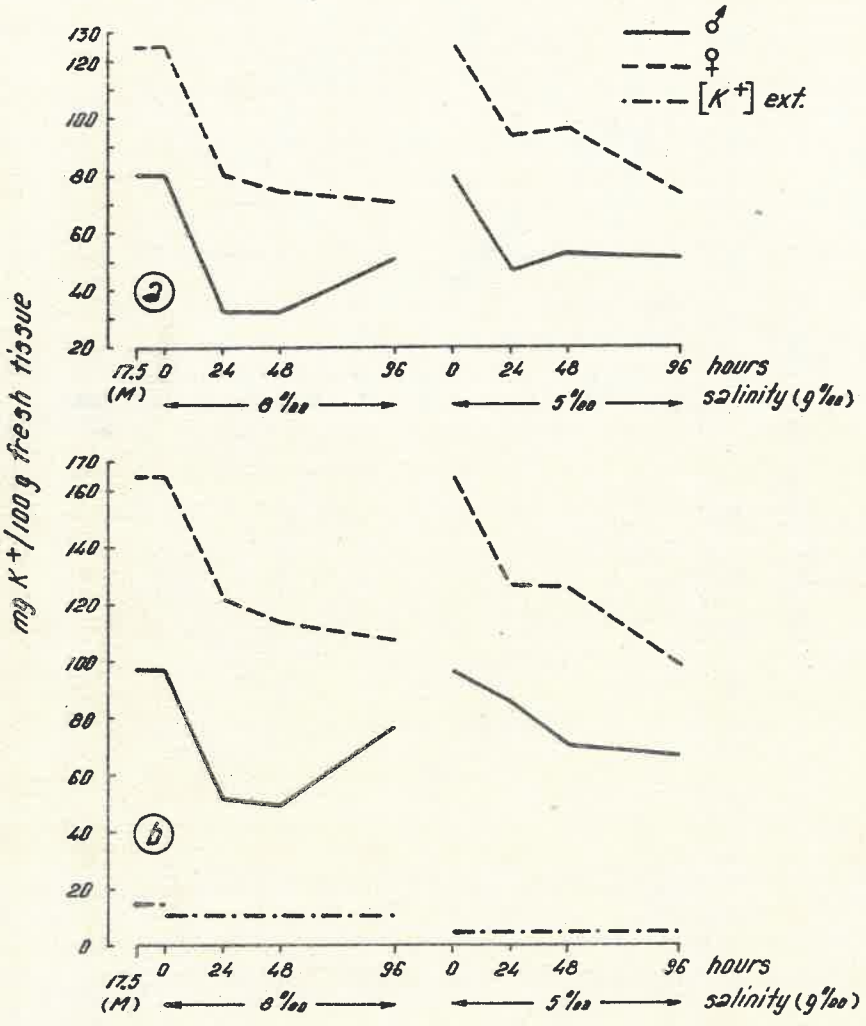

Fig.3 - Dynamics of K^+ ion in the body tissues of P.adspersus shrimp after the decrease of the salinity from 17.5‰ to 8 ‰ and 5 ‰ respectively; a - total tissular content; b - intracellular concentration

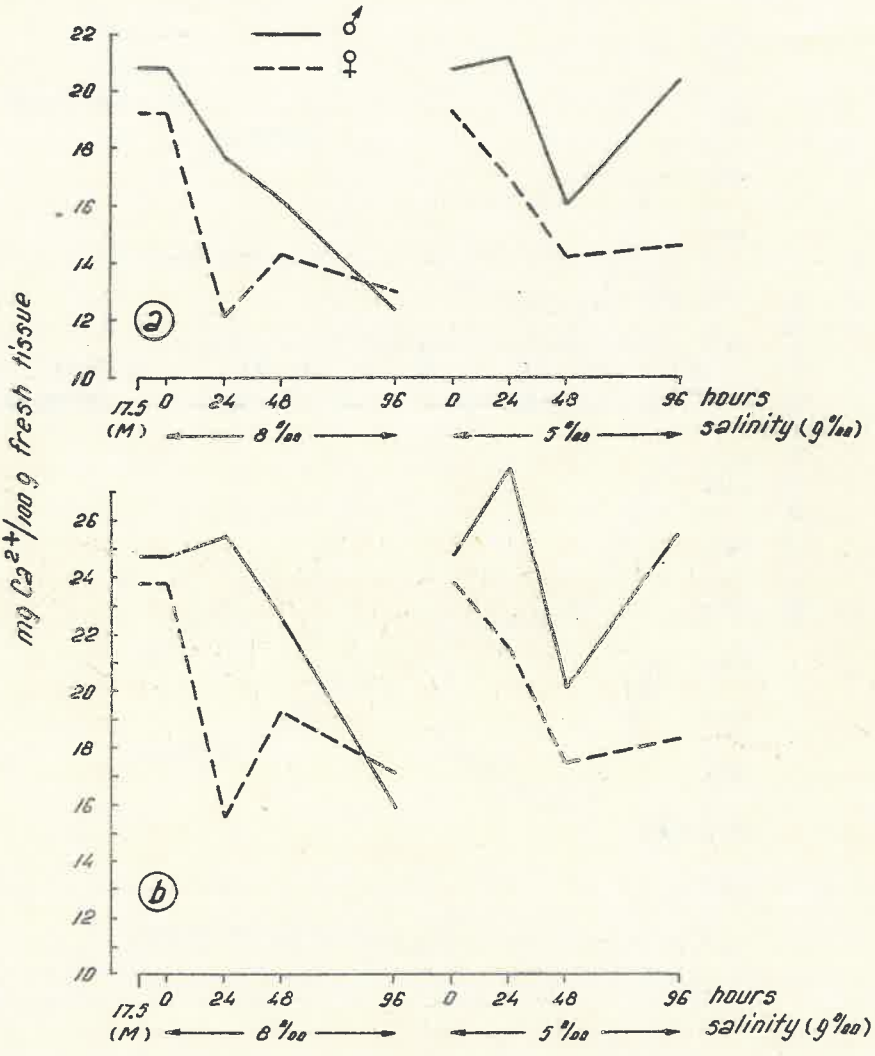

Fig.4 - Dynamics of Ca^{2+} ion in the body tissues of P.adspersus shrimp after the decrease of the salinity from 17.5 ‰ to 8 ‰ and 5 ‰ respectively; a - total tissue content; b - intracellular concentration of Ca^{2+}, including the calcium fixed in organites

The total amount of K^+ decreases drastically in the first 24 hours, because of the ion concentration decrease in the cells but subsequently, after 4 days, there comes out a visible tendency to restoration both quantitatively and especially its intracellular concentration (Table 1, Fig.3 a,b).

With respect to Ca^{2+}, its total content decreases gradually throughout the length of the experimental period. Also,its intracellular concentration decreases greatly,excepting the first 24 hours when an insignifiant increase was noticed (Table 1, Fig.4 a,b).

If the lowering in salinity of ambient medium is more advanced, namely from 17.5 ‰ to 5 ‰, the senses of changes in hydric content and ionic concenteations are likewise those depicted above, but the amplitude of phenomena is differed. Thus, after 24 hours, the gain of total water is something lesser advanced as well as the loss of water from the cells and its accumulation in extracellular space are less ample by comparison as observed to 8 ‰ salinity.

In the following 4 days hydremy continues to rise slowly, by keeping a high level in the extracellular space on the one hand and,by a gradual re-entering into cells, on the other (Table 1, Fig. 1 a,b,c).

The total amount in tissues Na^+ decreases because of a drastic going out of ion from the cells on the first day,and this low level remains (unalterable) throughout the experiment.

It is worth noticing however, that the intracellular level of Na^+ concentration remains higher than the extracellular content (Table 1, Fig. 2a,b). Concomitantly, we observe a continuous loss of potassium from the cells, having as result a decrease of total ion amount (Table 1, Fig. 3 a,b).

With respect to Ca^{2+}, there take place considerable fluctuations both quantitatively and of its intracellular concentration. Thus, after a day, Ca^{2+} concentrates into cells; subsequently, after 48 hours, its level decreases below the control and after 4 days returns a little over the control (Table 1, Fig. 4 a,b).

Table 2

The water and Na^+, K^+, Ca^{2+} dynamics across the cell membranes of the females of *Palaemon adspersus* in the low salinity conditions of the sea water

Salinity (g %o)	Time (H)	Water				Natrium			Kalium			Calcium		
		Water total (ml/ loo g f.t.)	W_e (ml/ loo g f.t.)	W_i (ml/ loo g f.t.)	W_e/W_i	$Na^+_{tot.}$ (mg/ loo g f.t.)	Concentration $Na^+_{ext.}$ (mg/ loo ml W_e)	Concentration $Na^+_{int.}$ (mg/ loo ml W_i)	$K^+_{tot.}$ (mg/ loo g f.t.)	Concentration $K^+_{ext.}$ (mg/ loo ml W_e)	Concentration $K^+_{int.}$ (mg/ loo ml W_i)	$Ca^{2+}_{tot.}$ (mg/ loo g f.t.)	Concentr. Ca^{2+}_{ext} (mg/ loo ml W_e)	Concentr. Ca^{2+}_{int} (mg/ loo ml W_i)
17.5	o	81.98	6.56	75.42	o.o8	271.72	44o.oo	322.o1	125.65	14.4o	165.36	19.26	2o.oo	23.8o
8.o	24	86.27	22.17	64.1o	o.34	133.94	2o1.oo	139.44	8o.27	1o.4o	122.56	12.17	9.5o	15.69
8.o	48	85.91	22.88	63.o3	o.36	158.45	2o1.oo	178.42	74.27	1o.4o	114.o6	14.32	9.5o	19.28
8.o	96	85.7o	22.94	62.76	o.36	165.73	2o1.oo	19o.6o	7o.41	1o.4o	1o8.38	12.96	9.5o	17.18
5.o	24	86.o4	12.42	73.62	o.17	62.83	66.oo	74.2o	94.o6	5.5o	126,84	17.o3	9.25	21.57
5.o	48	86.o7	1o.74	75.33	o.14	7o.77	66.oo	84.53	96.12	5.5o	126,81	14.2o	9.25	17.54
5.o	96	86.54	13.88	72.66	o.19	77.41	66.oo	93.93	72.61	5.5o	98.88	14.6o	9.25	18.33

2. THE EFFECTS OF DECREASED SALINITY ON HYDRIC AND MINERAL BALANCE TO FEMALES OF *Palaemon adspersus*

A first aspect as resulted from our experiments is that the females (wanting in eggs), in normal sea water (17.5‰ salinity) had a lower hydric content (total and intracellular) than the males; also, the total tissular content as well as the intracellular concentration of Na^+ were lower whereas for K^+, the values were far greater in comparison with the males (Table 2, Figs. 1,2,3).

The decrease to 8 ‰ of the sea water salinity had the same effects as to males but the amplitude of modifications was proportional to the level that sets out from the control. Thus, the total tissular water increased on the first day by its accumulation in the interstitial space with a simultaneously massif loss of it from the cells. Subsequently, in the following 3 days, the exit of water from the cells towards the extracellular space continued slowly,which results in the maintenance of a high total hydric level (Table 2, Fig. 1a,b,c). Concomitantly, takes place a decrease of intracellular Na^+ concentration which as early as on the first day reaches below the level from extracellular space. However,subsequently,a gradual increase of ion concentration into cells can be observed but that does not exceed after 96 hours,the value from interstitial space (Table 2, Fig. 2 a,b).

As concerns K^+, the decrease of both the total amount and the intracellular concentration was continuous and the tendency of return to the control value was no more observed as it was in the case of males (Table 2, Fig.3a,b). However, the value of intracellular concentration of ion remained higher than in the extracellular medium.

Concomitantly, a calcium loss from the cells took place (that affected the total amount of this ion in tissue, too) although the phenomenon oscillated since after 2 days an increase of its intracellular concentration was noticed (Table 2, Fig. 4a,b).

More dilution of sea water (from 17.5 ‰ to 5 ‰) also lead,to an increase of hydremy accompanied by a water gain in the extracellular compartment and a decrease of it in the cells (Table 2, Fig.1a, b, c), concomitantly with an obvious loss from the

cells of the main ions (Na^+, K^+, Ca^{2+}) which lead to their quantitative decrease from the tissues (Table 2, Figs. 2,3,4).

DISCUSSIONS

From the analysis of the results presented above, a first observation, comes out namely, in the normal sea water (17.5‰) the tissues which proceed from the males of P.adspersus contain, more water, Na^+ and Ca^{2+} and less K^+, in comparison with females. But, by decreasing the salinity of sea water to 8‰, entirely unexpected it comes out that on first day the cells lost a considerable quantity of water (from both males and females), the phenomenon being accompanied by a noteworthy decrease of Na^+, K^+ and Ca^{2+} concentrations.

In these new conditions of an environment with half the normal salinity,the boby fluids(the interstitial one inclusively) are hyperosmotics as compared with the external medium so that the water massively enters the extracellular compartment across the permeable surface (epithelia) of the boby, especially in the first 24 hours after the transfer of shrimp in a medium with low salinity (Fig.1b). Subsequently, on the following 3 days of the experiment, the tissular water balance is maintained steadfastly to the high level reached after the first day (Fig.1 a),because the exit of water from the cells is stopped, thus on males a slight tendency of the water to re-enter the cells can be observed as well as a gradual decrease of it from the extracellular fluid (Fig. 1 b,c).

The question which is rising now is to find out an explanation for the dehydration of cells in these new conditions of a hypo-saline medium when, in fact, it would have been normal to attend an entering of water into cells in accordance to the osmotic gradient.But,for this,it is necessary to have in view that, as early as the first hours after the transfer, the cells lost according to the new created gradient of concentration a considerable quantity of Na^+ of which concentration, at a given moment, is equalized to that from the extracellular medium and, moreover, is reduced even below this level (Fig.2b) and maintained to this new steady-state throughout the 96 hours of experiment.

With respect to potassium,its quick efflux on the first day, fails to equalize its concentrations at both sides of the membrane, remaining however, extremely slowly in the subsequent days for the females and entirely stopped for the males in which the ion begins to concentrate into cells (Fig. 3b).

To find a satisfactory answer at least to a part of the observed effects first of all the nature of the mechanism responsible for the uphill water efflux against the osmotic gradient which was ascertained in these experiments must be solved.

It is known that the water movement across the biological membranes can takes place by three distinct processes, two downhill of its concentration gradient (diffusion and filtration) and one by uphill water transfer.

The filtration transfer coresponds to a movement by "bulk flow", the driving force being the osmotic pressure of fluids separated by membrane. Experiments on artificial membranes have proved that the water solutes exert a negative pressure which direct the water from hypo-osmotic compartment to the hyper-osmotic (4). This, however, cannot justify the water efflux from the cells, ascertained by us, since the intracellular medium was hyper-osmotic in comparison with the extracellular.

On the other hans, the diffusion, on the basis of some thermodynamic considerations, assumes that water movement across biological membranes takes place downhill its concentration gradient too, not through wide pores to allow the bulk flow but through small molecular pores. Support for this view is the so called "the solvent-drag effect" (1) that assumes the existence of an osmotic flux and consists in the speeding of the solute flux in the direction of the water movement and a simultaneous reduction on the opposite direction. However, our experimental results have shown that there is no osmotic efflux of water since the movement takes place uphill the osmotic gradient. The sole explanation to justify the apparently abnormal water loss from the cells observed by us, can be offered by "the pump-leak model" (6). In accordance with this model, if an ion is actively transported and maintained in excess outside the cell, it can balance against an excess of intracellular osmotic pressure.All the more, if the active transport of this ion will be stimulated in one way

or another, its storage in extracellular compartment will drag-along across the membrane, a part of intracellular water, so diminishing the cell volume.

The explanation for such apparently abnormal water movement uphill the osmotic gradient, was suggested too, by others who showed that the ions transported actively outside the cell are able to "drag along" water molecules by a process similar to the solvent drag (8).

Considering the phenomena found out by us, namely, even on the first day after transfer of shrimps in water of 8 ‰ salinity, the intracellular concentration of Na^+ decreased more than the extracellular one was lowered by dilution of sea water and, on the other hand, the fact that potassium intracellular concentration is maintained higher than the extracellular, then we can admit the involvement of an active transport of the two ions, stimulated in these news conditions. In other words, an activation of Na^+-K^+ pump as early as the first day after the transfer takes place, the efficiency of this mechanism maintains the normal sense of the concentration gradients for Na^+ and K^+ all the time, but to a new steady-state level.

The stimulation of the active transport for Na^+ and K^+ by an intensification of ATP-ase activity, in media of lower salinity, was already proved experimentally by KAMEMOTO and TULLIS (5) in studies on the kidney of Procambarus clarkii and on gills of Metopograpsus messor but the mechanism responsible for this stimulation has not been cleared up yet. However, there are some studies which suppose the existence of a feed-back regulator mechanism which allows the readjustement of water permeability of the body surface in response to the salinity modifications. Thus, LOKWOOD et al. (7) show that in Gammarus duebeni, the modifications of permeability, measured by HTO turnover, appear in five minutes when the animals adapted to sea water were transferred in dilute media. The authors suggest that the stimulus initiating the changes of permeability is not an osmotic one, thinking that there are two possibilities: either a nervous control of the permeability, or a direct effect of ions on the membrane permeability.

The salinity rather than the osmotic pressure should be the stimulus which interferes on the receptors (2).

However, the existence of more other intervening mechanisms cannot be excluded. Among these, surely the Ca^{2+} concentration has an important role. It is generally accepted that in the extracellular medium, calcium ion has a "stabilizing" effect on the membrane permeability (9). Moreover, the Ca^{2+} intracellular concentration affects the ionic movements too, any increase of its concentration leading to the inhibition of Na^{+}-K^{+} pump on the inside of the membrane like ouabain on the outside of it (3).

In view of our experimental results where by lowering of salinity to 8 ‰, both intracellular calcium concentration (Fig. 4b) and its content (Tables 1 - 2) decreased significantly (to males and females), it can be admitted that in these new conditions the calcium inhibitory action on the Na^{+}-K^{+} pump should be reduced. In this way, the loss of intracellular water by the so called "pump-leak mechanism", uphill the osmotic gradient appears justified.

Whenever the salinity of ambient medium was even more reduced to 5‰, the water loss from the cells was smaller (Fig.1c); in exchange, the natrium loss (Fig. 2b) was more pronounced in comparison with the 8 ‰ salinity of external medium.

The higher natrium intracellular concentration than the extracellular one can be justified by a lower efficiency of the pump activity, that its working capacity is now overrun and it fails to restore the normal sense of the concentration gradients of the two ions actively transported (Na^{+} and K^{+}).

However, since the intracellular potassium concentration remained higher than the extracellular and natrium intracellular concentration drastically lowered (especially to females) still on the first day and continued so, in these limits, throughout of experiment, it can be admitted that, even in these conditions of an intense dilution of sea water, the shrimps still have the mechanisms which can allow them to regulate their cell volume.

CONCLUSIONS

1. In the normal sea water (17.5‰ salinity) the tissues which proceeded from the males of P.adspersus contained more water, Na^{+} and Ca^{2+} and less K^{+}, in comparison with the females.

2. After the transfer of shrimps in sea water having 8‰ salinity, the cells lost water, Na^+, K^+ and Ca^{2+}.

3. The water loss uphill the osmotic gradient is explained by "pump-leak" hypothesis.

4. The senses of ionic gradients for Na^+ and K^+, to 8‰ salinity, are kept normally but to a new steady-state level imposed by pump activity.

5. To 5‰ salinity of environment, the senses of phenomena were similar to those observed at 8‰ but of lower amplitude, excepting the more amplified Na^+ efflux. Moreover, the normal sense of Na^+ gradient is not recovered.

6. In hypo-saline media, both the males and females of P.adspersus showed the osmoregulation possibilities accountable both by modifications of the passive permeability of cell membranes and by a stimulation of the active ionic transport mechanisms.

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