# Organic 'compensatory' osmolytes in osmolarity control and hydration changes in animal cells

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This paper briefly reviews some aspects of the research on organic solutes in osmolarity control and hydration changes of animal cells. It is concerned with the nature of these solutes, their roles in acclimation to hyperosmotic situations, the control of their concentration, and the sensing and signalling of osmolarity changes.

## 1. Cell osmoregulation and activity of macromolecules

Cells can be viewed as highly sophisticated assemblages of macromolecular systems, the activities of which are strictly dependent on a few basic requirements. These include a proper spatial organization and a proper concentration in their immediate environment of the solutes they can interact with. In this framework, cell osmoregulation can be considered as dealing with the various mechanisms evolved at the cellular level to meet these requirements. Water is indeed the physical medium in which most of the interactions between solutes and macromolecules take place. Its availability depends only on its osmotic movements and, further, its mobility in cell systems is always far greater than that of the different compounds acting as osmotic effectors. The control of the concentration of these osmolytes thus becomes essential in the control of the osmotic movements of water across cell boundaries and, therefore, in the control of the spatial organization of macromolecules and of the level of interacting solutes in their vicinity.

In animal cells, which lack rigid and highly resistant walls, cell osmoregulation is closely related to cell volume maintenance. It tends to keep the intracellular osmolarity extremely close to isosmotic equilibrium with the environmental medium, even during changes in its osmolarity. Thus, any increase in environmental osmolarity will induce a similar increase in intracellular osmolarity and vice-versa.

## 2. Cell osmoregulation and changes in blood osmolarity

Changes in osmolarity of the cellular environment may occur in a variety of conditions. These include changes in blood osmolarity in many species experiencing changes in the availability or in the salinity of their environmental water. Many examples of such changes have been presented elsewhere (Gilles & Delpire 1997). For instance, increases in blood osmolarity of 500 mOsm/l and more are currently reported in euryhaline invertebrates and primitive fish (worms, molluscs and crustaceans, agnathes and selacians: from ± 500 to ± 1000 mOsm/l) going from fresh or brackish water to sea water or passing from pools of diluted to normal or even concentrated sea water. Changes of about 30% in blood osmolarity have also been reported in aquatic euryhaline higher vertebrates experiencing increases in external salinity (teleosts, fish, turtles: from ± 300 to ± 400 mOsm/l). In terrestrial species, changing the salinity of the available drinking water may also lead to significant change in blood osmolarity. In California and Gambel's quails, for instance, changing the drinking water from tap water to 50% seawater induces an increase in blood osmolarity from 350 to 480 mOsm/l. Water shortage, when of long duration, may also lead to more or less pronounced dehydration with a concomitant increase in blood osmolarity. Desiccation limits can be quite high in invertebrates as well as in vertebrates up to reptiles, ranging from 20 to 60% of the total body water without apparent damage. Up to twofold increases in blood osmolarity are currently reported in these situations. Most mammals and birds do not tolerate large decreases in extracellular water volume. They become seriously affected after losing 10 to 15% of their body water. Desert species such as camels and goats do a little better since they can lose twice that amount without showing any apparent weakness. In these situations, blood osmolarity may increase by 30 to 60 mOsm/l.

Extracellular fluid osmolarity may also change considerably in a variety of secretion and absorption processes. The best known example of this is the concentrating mechanism of the loop of Henle in the mammalian kidney. In the process of urine formation, the osmolarity of the papillary cells at the tips of the loops may vary from 700 to 3000 mOsm/l or more, depending on the species.

In all these situations, the cells have to cope with changes in osmolarity of their surrounding fluids; the survival of species in such conditions is thus strictly dependent on the ability of the cells to face these changes in osmolarity successfully.

## 3. Cell osmotic balance: organic and inorganic osmotic effectors

Hundreds of references dealing with osmotic effectors in metazoan cells are available in the literature. Reviewing the question of cell osmotic balance with some accuracy remains, however, a major problem, most studies having essentially dealt with one or only a few selected compounds. Moreover, results are expressed in a variety of ways, making any quantitative comparison impossible (tissue wet weight, number of cells, mg proteins, mmoles per kg intracellular water...). Often the results have been obtained in completely different conditions, experiments dealing with short term osmolarity changes applied in different ways and cells trying to 'acclimate' to them, or with long-term changes and cells 'accli-

mated' to them. It is thus very difficult to get a clear idea of the role of a given compound in the cell osmolarity adjustments.

Considering the problem in general terms, some basic statements can, however, be made. (a) In all cell types, the inorganic ions $Na^+$, $K^+$ and $Cl^-$ are important osmotic effectors. In most cases, $K^+$ is far more concentrated than $Na^+$ or $Cl^-$. Considering that a substantial part of it is free and, therefore, osmotically active, $K^+$ is certainly the major inorganic cell osmotic effector. (b) In all cell types, different organic compounds play some part in the osmotic balance. Their importance depends on the species considered. In the vertebrates, with the exception of agnathes and selacians, cellular osmolarity is generally low (around 280–350 mOsm/l) and the inorganic ions account for most of it (between 200 and 300 mOsm/l). The rest is essentially made up by different organic osmolytes of low molecular weight which account only for some 50 to 100 mOsm/l (Figure 1). The situation is about the same in freshwater invertebrates. It is quite different in marine invertebrates and primitive fish. In these cases, the osmolarity is more than three times higher. Of the 1000 mOsm/l of the intracellular osmolarity, some 200 to 300 can be accounted for by the inorganic ions $Na^+$, $K^+$ and $Cl^-$, as in the vertebrates. The rest of it (700–800 mOsm/l) is made up by different organic molecules, among which different amino compounds and sometimes polyols play a prominent part. In other species (mostly elasmobranchs and sarcopterygian fish but also amphibians and reptiles) and tissues (mammalian kidney medulla), urea is also used as a major osmotic effector (Figure 1). (c) Interestingly, the intracellular amount of the major inorganic osmolytes ($Na^+$, $K^+$ and $Cl^-$) remains about the same in these different situations in spite of the fact that their concentration in the extracellular medium varies greatly. In animal cells, thus, the inorganic ions always account for roughly the same amount in the total intracellular osmolarity. The rest, whatever its importance, is always made up by different organic compounds

This also seems to be true in a single cell type withstanding changes in the osmolarity of its external medium. The examples in Figure 2 are a marine invertebrate cell, a mammalian cultured cell and a kidney medulla cell. These results clearly indicate firstly, that acclimation to high osmolarity involves little or no change in inorganic ion levels; this is confirmed by a series of measurements obtained on different renal cell lines in the late eighties (Bagnasco, Balban, Fales, Yang & Burg 1986; Nakanishi, Balban & Burg 1988), and secondly, that the increase in cell osmolarity is essentially related to an increase in the amount of other compounds, some of which remain undetermined, but among which a few organic osmolytes always play a prominent part.

The fact that the cell inorganic ion levels do not change significantly or change only slightly, while the amount of NaCl in the extracellular medium is greatly increased, indicates regulation of mechanisms involved in the control of the

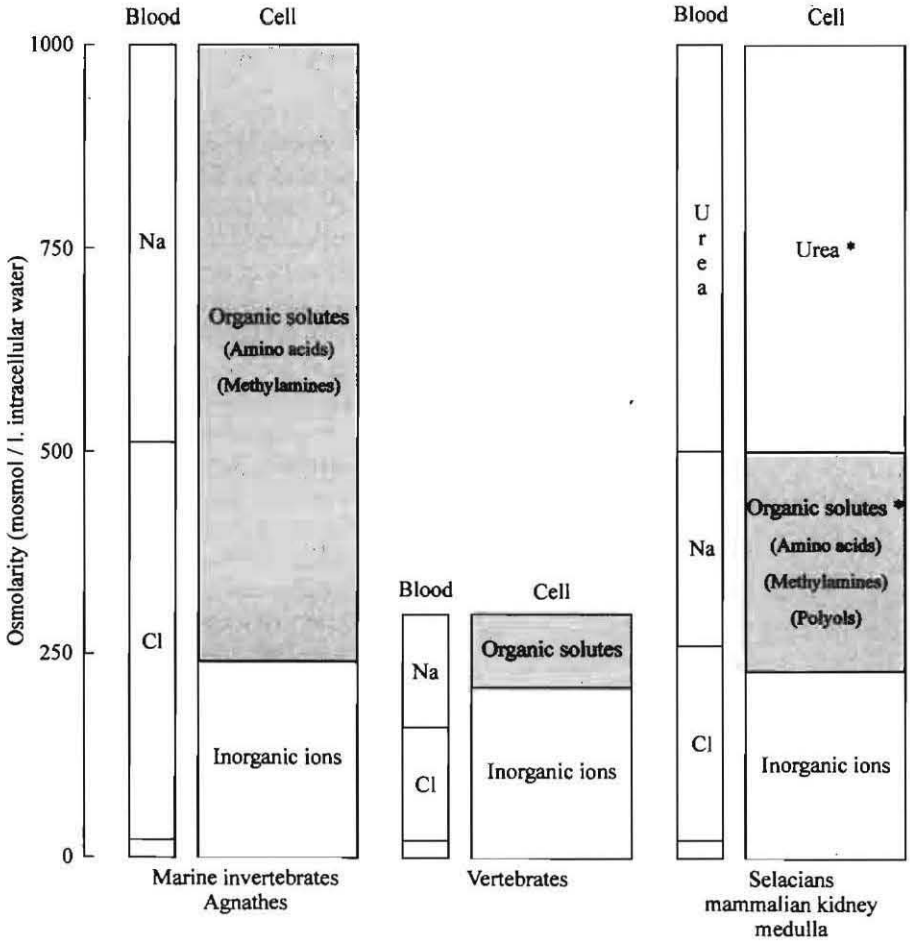



**Figure 1** Major osmolytes and idealized osmotic balances in cells and blood of different animal groups. * Urea in mammalian kidney is highly variable depending on the diuretic status. However, it always distributes similarly in blood and tissues.

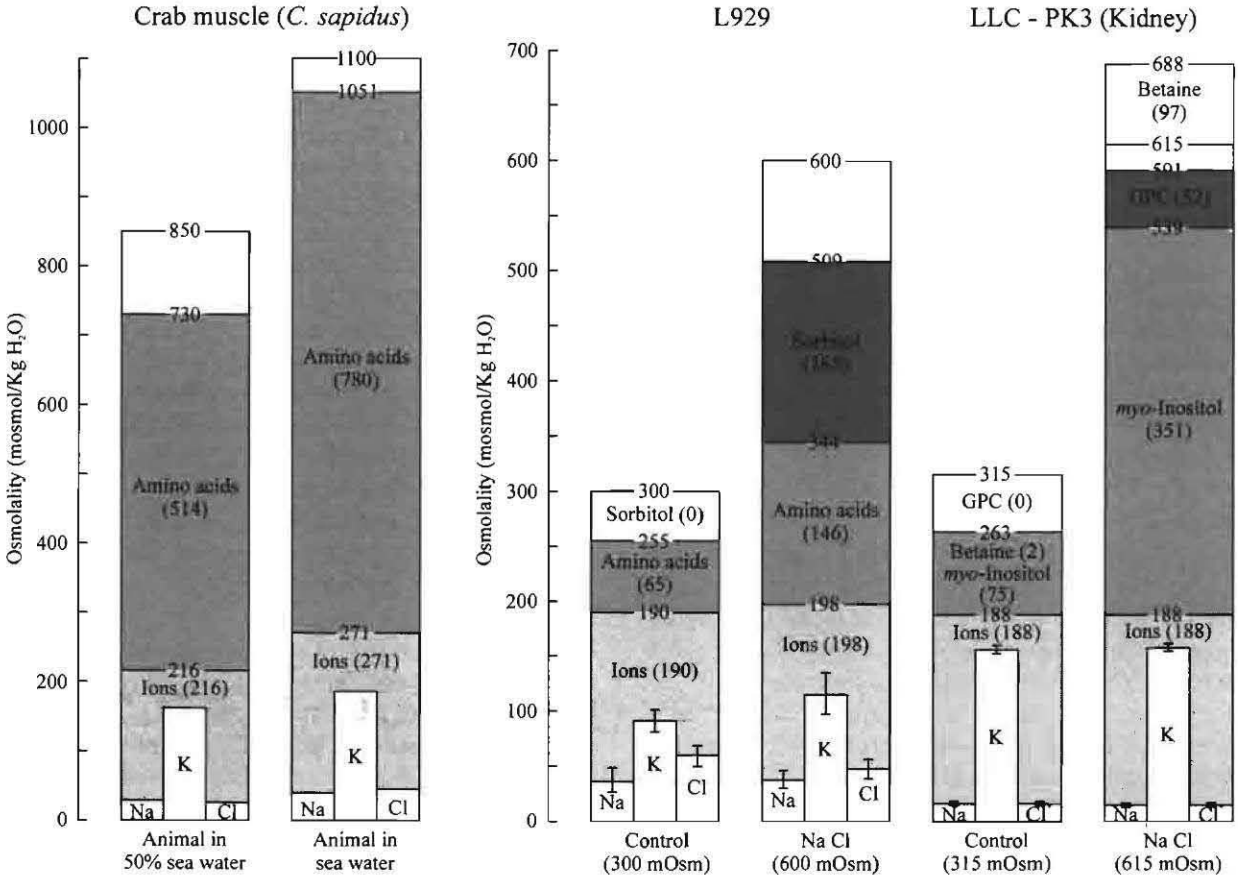



**Figure 2** Osmotic balances in different environments of cells from a marine invertebrate and from different mammalian origins: L929 (subcutaneous tissue) and LLC-PK3 (kidney medulla). GPC: glycerophosphorylcholine. Crab muscle data are from Gérard & Gilles 1972; L929 from Libioulle *et al.* 1996; LLC-PK3 from Nakanishi *et al.* 1988.

intracellular content of these ions. To our knowledge nothing has yet been done in that field. Most research has up to now concentrated on the organic osmolytes, trying to answer the following major questions. What are these compounds and what is their role? How are they accumulated? What are the primary triggering mechanisms of their accumulation? Would cells loaded with such compounds become more tolerant to osmotic shocks or to other stresses related to intracellular water availability?

Let us now briefly consider these different problems.

## 4. Nature of the organic osmolytes

To our knowledge, the first identification of a major group of organic solutes related to tissue osmotic adjustments was published by Duchateau & Florkin in 1955. Their report dealt with free amino acids in the muscle tissue of a euryhaline marine decapod, the Chinese crab *Eriocheir sinensis*. It showed that the muscle concentration of these compounds was approximately doubled on acclimation of animals from fresh water to sea water.

Since this first report, hundreds of studies dealing with organic osmolytes in a variety of animals, tissues and cell types have been published, essentially from the early sixties to the mid-eighties. These results have been reviewed from time to time (see for instance Gilles 1979, 1987a, b; Yancey, Clark, Hand, Bowlus & Somero 1982; Borowitzka 1985; Nakanishi *et al.* 1988; Anthoni, Christophersen, Hougaard & Nielsen 1991; Gilles & Delpire 1997). To make a long story short, let us briefly say that: (a) a variety of compounds have been identified as major organic osmolytes in animal cells (Figure 3). Their occurrence and amount vary from species to species, in a single species from tissue to tissue and even in a single tissue from cell type to cell type as has been demonstrated in a survey of osmolytes in renal cell lines (Nakanishi *et al.* 1988). (b) Some amino acids, particularly non-essential ones such as proline, glycine, aspartate, glutamate, serine and alanine are of much importance in most marine invertebrates. Other amino compounds such as taurine or different methylamines including glycine betaine and trimethylamine oxide (TMAO) are also generally found (TMAO is usually reported in association with urea in species using this compound in their osmotic balance). Most of these compounds are also found, in much lower concentrations of course (refer to Section 3), in vertebrate tissues. Glycerophosphorylcholine (GPC), myo-inositol and sorbitol have also been reported in a variety of mammalian cell lines; they play a major role, together with betaïne, in the osmotic adjustments of the renal medulla cells (Beck Dörge & Thurau 1988; Nakanishi *et al.* 1988; Yancey 1988).

## 5. Roles of the organic solutes – osmolytes: compatible, counteracting and compensatory

The different organic solutes found in large amounts in tissues were first and *de facto* considered as osmotic effectors. In his pioneer work on invertebrate species acclimated to different salinities, Marcel Florkin (1962), considering that there

## Amino compounds

### Amino acids

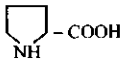
Carboxylic

Alanine $CH_3-CH(NH_2)-COOH$

Aspartate $HOOC-CH_2-CH(NH_2)-COOH$

Glutamate $HOOC-CH_2-CH_2-CH(NH_2)-COOH$

Glycine $H-CH(NH_2)-COOH$

Proline 

Serine $CH_2(OH)-CH(NH_2)-COOH$

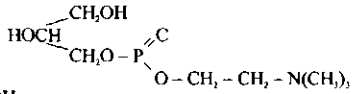
Sulfonic

Taurine $SO_3H-CH_2-CH(NH_2)$

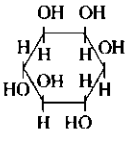
### Methylamines

Glycine betaine $(CH_3)_3N-CH_2-COOH$

Trimethylamine oxide $(CH_3)_3N=O$

Glycerophosphorylcholine 

### Polyols

*myo*-Inositol 

Sorbitol $CH_2OH-(CHOH)_4-CH_2OH$

**Figure 3** Major organic osmolytes in animal cells.

were only slight modifications in tissue hydration during acclimation, proposed 'to consider the variation in the amino acid component resulting from a change in the medium concentration as exerting an intracellular osmotic regulation'.

Insight into another role of organic osmolytes came from a study of the participation of different polyols in the osmotic equilibrium of an osmophilic yeast by Brown and Simpson. In a basic paper of 1972, they reported that glycerol did not affect the activity of an enzyme extracted from the yeast. This led them to consider a notion of 'compatibility' with protein function. They stated 'a compatible solute may be loosely defined as one which, at high concentration, allows an enzyme to function effectively'. This notion was reintroduced in the literature on animal cell osmoregulation in the late seventies by Somero and his colleagues (see for instance Bowlus & Somero 1979). In the same line of thought, the terms 'stabilizing' or 'nonperturbating' solutes can also be found (see for instance Clark 1985, 1987). Another interesting idea was also developed at about the same time by the Somero group after the demonstration that some stabilizing compounds such as trimethylamine oxide can offset the destabilizing effect of urea on enzyme function (Yancey & Somero 1979). Trimethylamine oxide is usually found in species such as elasmobranchs using urea as a major osmotic effector. In 1982, in an excellent paper signed by the whole group, the term 'counteracting' was proposed to name these organic osmolytes (Yancey *et al.* 1982). Interestingly, Yancey demonstrated more recently that this 'counteracting' effect on urea also has an important physiological meaning in mammalian kidney cells (Yancey & Burg 1990).

In 1981, Mary Clark showed that the methylamines can offset the perturbating effects not only of urea but also of inorganic ions (Clark, Hinke & Todd 1981). On that basis, she introduced the term and concept of 'compensatory' solutes. She stated 'not only are some solutes (KCl, NaCl) deleterious to macromolecular integrity, while others (neutral and acidic amino acids) are innocuous, but in addition, some solutes can, in fact, offset or compensate the effects of others; there is an algebraic additivity among solutes' (Clark 1985).

I adopted the term 'compensatory' in 1987 reviews (Gilles 1987a,b) on the grounds that amino-compounds can not only oppose the disrupting effects of ions on macromolecular structures but also that they somehow 'allow a regulation of the intracellular concentration of inorganic ions back to values close to control' (Gilles 1987b), as pointed out in Section 3 above. In this view, organic osmolytes appear thus as 'compensatory' for the osmotic gap left due to maintenance of steady levels of inorganic ions. This role, generally overlooked up to now, is of much importance since it prevents the increase in inorganic ions to disrupting levels that would otherwise occur in hyperosmotic conditions (see also Gilles, 1997). Figure 4 illustrates the compensatory effect of organic osmolytes on the inhibition of enzyme activity by high salt levels and on the precipitation of chromatin caused by high salt levels. Interestingly, the effect of high inorganic ion levels on enzyme activity is not always negative. Depending on the enzyme considered, there can indeed be no effect, inhibition or activation. In some cases, as seen in Figure 5, the effect can go from inhibitory to activatory depending on the conditions. These differences indicate that the situation is probably more complex than usually realised. They could also be of much physiological meaning; they can indeed be integrated into a metabolic regulation scheme that could play a part in the adjustment of the level of some osmolytes of the amino-acid group (see Section 6, below).

Compensatory effects on isolated enzymes have been described by many groups over the past 25 years and on isolated chromatin by our group starting in 1989 (see Figure 4). Could they be demonstrated on intact cells submitted to osmotic shocks? To our knowledge, this has never been specifically looked for in enzyme activities and metabolic pathways. Metabolic effects of acclimation to media of different osmolarities are, however, well-known (see for instance Haussinger, Gerok & Lang 1993 for a recent review on hepatic metabolism). As far as chromatin is concerned, we discovered in 1985 that it undergoes massive condensation and decondensation in mammalian cells and tissues submitted to abrupt hyper- or hypo-osmotic shocks (Delpire, Duchene, Goessens & Gilles 1985). Such changes are barely observed in tissues of marine euryhaline invertebrates which have high intracellular levels of amino acids and other compensatory organic osmolytes, as discussed in Section 3 (Gilles & Goffinet 1991). Similarly, mammalian L929 cells preloaded with sorbitol, or preacclimated to a high NaCl medium which

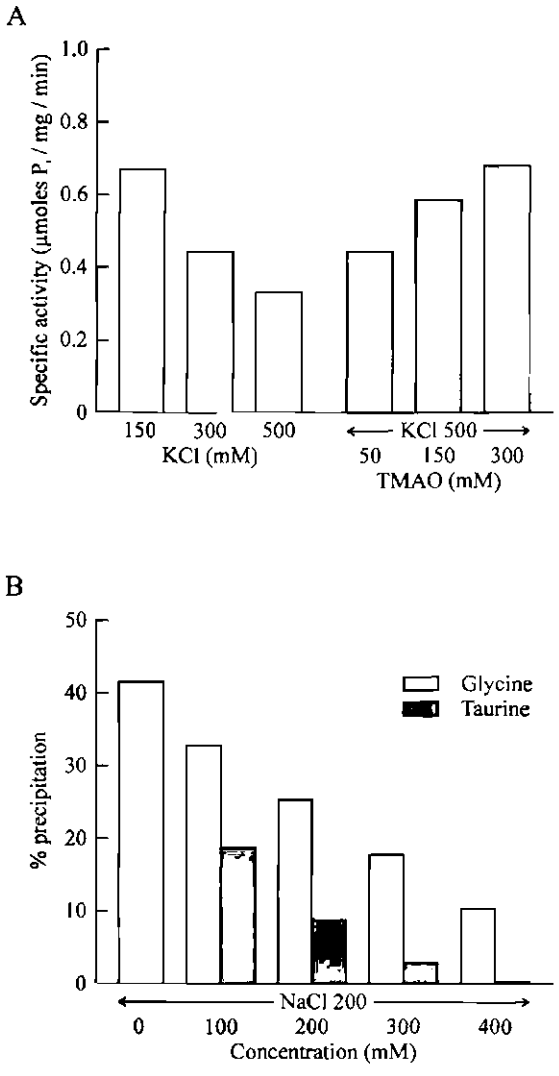



**Figure 4** Examples of 'compensatory' effects of organic osmolytes on the disrupting effect of NaCl on: A: activity of $Ca^{2+}$-activated myosin ATPase from rabbit muscle (after Clark 1985, modified); B: precipitation of native chromatin isolated from PC12 cultured cells (after Buche, Ouassaidi, Hacha, Delpire, Gilles & Houssier 1989, modified).

increases the intracellular amount of organic osmolytes, do not show chromatin hypercondensation when submitted to an abrupt increase in medium NaCl (see Figure 6). These effects can be easily interpreted considering that application of an abrupt hyperosmotic shock induces a large cell shrinkage and therefore an increase in the level of the inorganic ions (see Clegg 1988 and also Figure 10) to disrupting levels, inducing structural changes responsible for the hypercondensation of chromatin. Such effects are obviated by the presence of compensatory osmolytes. With this in mind, it is interesting to consider that the precipitating effect of high NaCl on isolated chromatin can be 'compensated' by the presence of different organic osmolytes (Figure 4).

This brings us to the next two problems I would briefly like to consider. How is the amount of the compensatory solutes regulated and would they be active in stabilizing macromolecules in other 'ionic stress' situations?

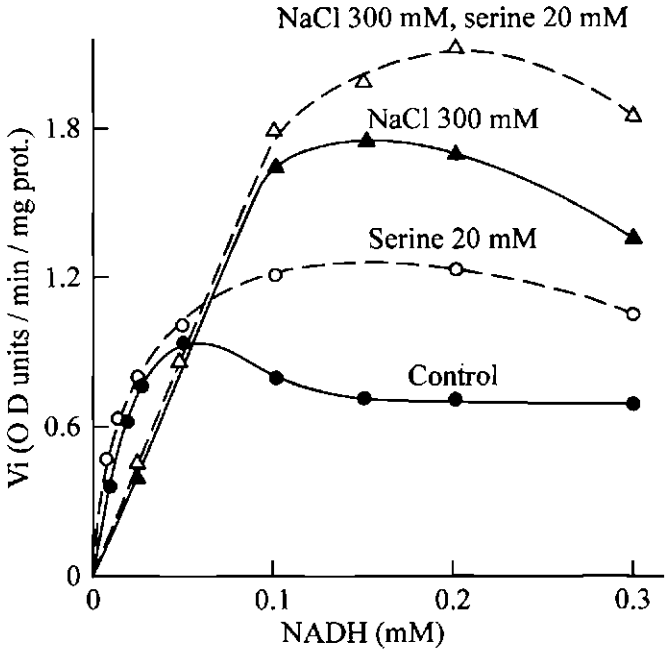



**Figure 5** Effect of NaCl and serine on the activity of glutamate dehydrogenase from the muscles of the euryhaline crab *Eriocheir sinensis* (after Gilles 1974, modified).

## 6. Regulation of the concentration of compensatory compounds

The studies in this field run from the early sixties up to now. It is actually clear that the control of the levels of the different compensatory solutes cannot be related to a single major mechanism. Changes in fluxes (in and/or out — transport and permeability) as well as in metabolism (catabolism, *de novo* synthesis, oxidation to $CO_2$) can be implicated, depending on the solute considered and on the conditions in which the cells are. It is actually impossible to produce an integrated view of how these changes are controlled. Control of the amount of one single solute may involve more than one process. Cells may also change major solutes and/or accumulation mechanisms depending on the conditions (availability in the external medium, in particular). We will thus refer the reader to the available literature on specific solutes and specific cell types whenever it exists.

*A priori*, control can be exerted in different ways: (a) directly, by one or several 'modulating factors' acting on target systems: transporters, channels and/or enzymes. Such an 'allosteric' modulation would lead to changes in kinetic characteristics of the target system(s). (b) Indirectly, by regulation of the expression of genes coding for the synthesis of the transporters, channels and/or enzymes implicated. In this case, there would be a change in the concentration and eventually also in the kinetic characteristics of the target systems. One system is evidently not exclusive of the other. They could even go quite nicely together since genic regulation is much slower than allosteric modulation. It is of interest to consider in this framework that induction by osmotic challenges of transporters and enzymes usually takes at least a few hours and often one day or more (see for instance Libioulle, Llabres & Gilles 1996). An allosteric control could thus be at play in the early steps, immediately following applica-

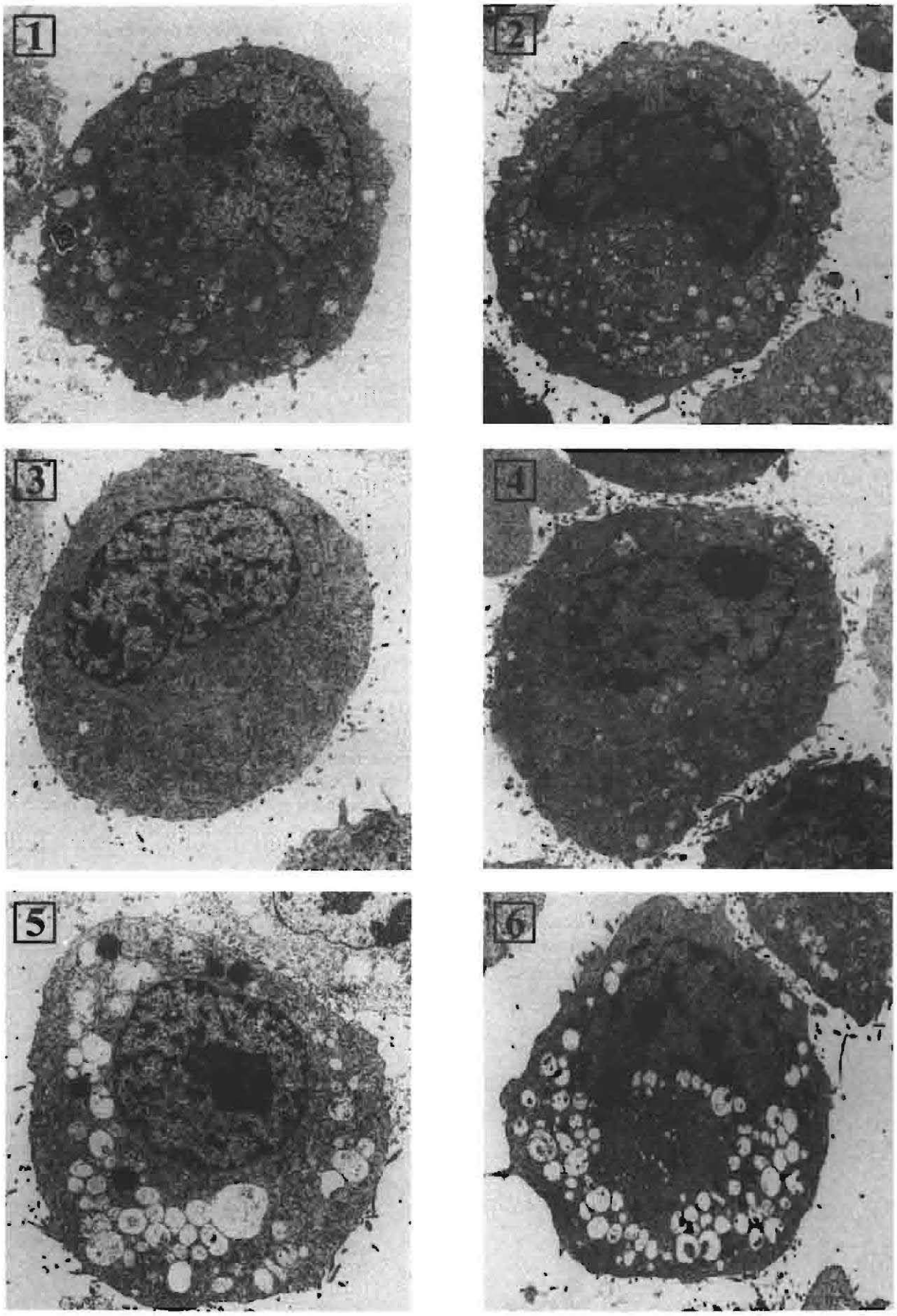



**Figure 6** Effect of a NaCl hyperosmotic shock (+ 154 mM NaCl) on L929 cells in control conditions (1, 2) or preacclimated to 600 mOsm media by addition of NaCl (3, 4), or sorbitol (5, 6) — 1, 3, 5 before, 2, 4, 6 after application of the hyperosmotic shock. (After Gilles *et al.* 1995, modified).

tion of osmotic shocks, while genic regulation would become effective later on, in long-lasting changes in external osmolarity. Interestingly, both systems would have to work in quite different conditions: the early steps of cell osmotic adjustment are often concomitant with changes in volume and therefore in intracellular solute concentrations, notably in $Ca^{2-}$ and in the major inorganic ions.

In this framework, the study of the direct effects of inorganic ions and of $Ca^{2+}$ on the activity of enzymes and transporters implicated in the control of the level of organic compensatory solutes would be of much interest. This idea has not been developed much up to now. As far as I know, I have been the only one to consider it in the sixties and to integrate the effects of ions on the activity of different enzymes in a metabolic sequence (Figure 7) that could account for the adjustment of the amount of some amino acids in the tissues of euryhaline invertebrates (Gilles 1969, 1974, 1978). Such studies, including effects of $Ca^{2+}$ on other enzymes and transporters, would in my view be most welcome and, possibly, rewarding.

Major arguments favouring the idea of indirect control though regulation of gene expression emerged from microbiological studies on the control of proline and glycine-betaine transport by the end of the seventies. Such studies then developed on animal cell models, starting in the mid-eighties, essentially with Maurice Burg as steering influence. Most of this literature has been reviewed from time to time (see for instance: Garcia-Perez & Burg 1990, 1991a,b; Burg Garcia Perez 1992; Burg 1994; Kwon & Handler 1995; Cohen 1997; Handler & Kwon 1993, 1997; Gilles & Delpire 1997). We shall thus not dwell on these studies here and refer the interested reader to these reviews. They can be usefully summarized in a scheme (Figure 8) showing that an increase in external osmolarity will somehow, through a signalling cascade, activate osmotic response elements (ORE) on the genes coding for enzymes or transporters implicated in the control of the intracellular level of osmolytes. Research on the signalling cascades and on the OREs is developing quite rapidly. A major question left in this scheme is, in our view, the primary sensing process. How can a cell know about the osmolarity of its environmental medium? What makes it adjust the activity of its osmogenes?

## 7. Primary sensing of osmolarity changes

On the basis of the mechanisms described in yeasts and bacteria, a change in cell inorganic ion levels would be a clear candidate as primary triggering system. In these microorganisms, expression of osmogenes (genes sensitive to osmotic changes) correlates positively with the accumulation of $K^+$ that occurs as a primary response to hyperosmotic conditions. As far as animal cells are concerned, Uchida, Garcia-Perez & Burg (1989) studied the induction of aldose reductase by hyperosmotic shock in a mammalian kidney cell line. Increase in the level of that enzyme is responsible for the accumulation of sorbitol, a major organic osmolyte of these cells. Relating the $Na^+$ and $K^+$ level changes occurring in their experimental conditions to the increase in enzyme level, Uchida et al. suggested that 'the signal that triggers aldose reductase induction most likely is increased intracellular ionic strength'. Much current research is polarized on that idea, and a variety of recent results indicates that hypertonicity would stimulate a kinases cascade which in turn would activate osmogenes via osmotic response elements (Figure 8; Itoh, Yamauchi, Miyai, Yokoyama, Kamada, Ueda & Fujiwara 1994; Terada, Tomita, Homma, Nonoguchi, Yang, Yamada, Yuasa, Krebs, Sasaki & Marumo 1994; review Cohen 1997). All these studies, however, deal with stress conditions, looking for changes occurring right after an 'abrupt' application of the hyperosmotic medium. As already stated, cells in such a situation undergo important shrinkage and dehydration with a concomitant increase in intracellular solute levels. The physiological meaning of the changes observed may thus be questioned. In mammalian systems *in vivo*, changes in osmolarity of the external medium occur only slowly. Further, the large ratio of intracellular to extracellular volume should oppose the development of large osmotic gradients (see Gilles & Delpire 1997 for discussion). We recently studied the inorganic ion levels together with aldose reductase induction in L929 cultured cells submitted to hyperosmotic media in different conditions: long term acclimation, abrupt osmotic shock or slow increase in osmolarity. In these cells, sorbitol is by far the most important organic osmolyte (Figure 2, Libioulle *et al.* 1996), and they can be easily acclimated to media made hyperosmotic not only by addition of NaCl but also of various organic compounds such as sorbitol or proline

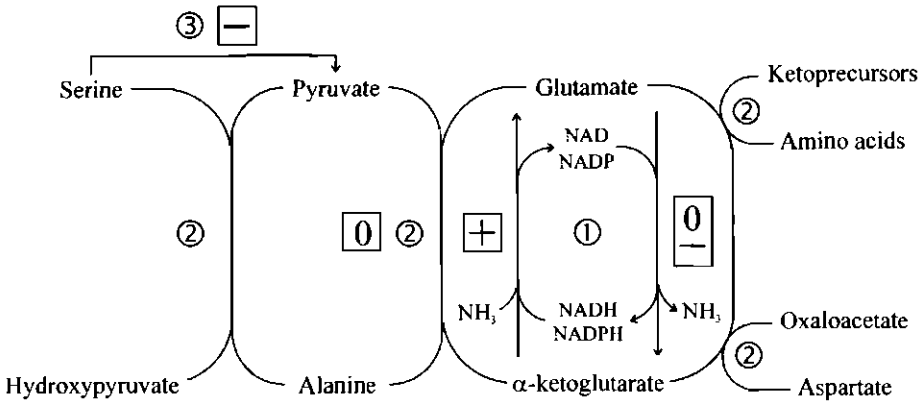



**Figure 7** Effect of an increase in NaCl on the transdeamination sequence controlling the level of some amino acids acting as major osmolytes in animal cells. 1: glutamate dehydrogenase, 2: transaminases, 3: serine hydrolase; +: activation, –: inhibition, O: no effect (after Gilles, 1969, 1974, 1978).

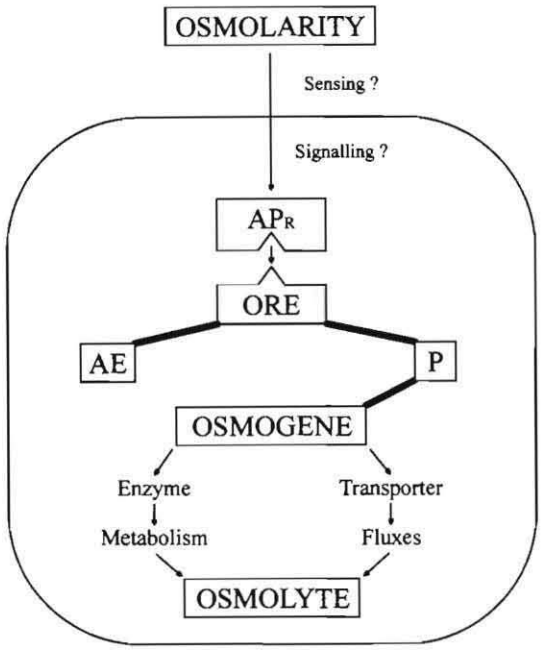



**Figure 8** Control of organic osmolyte levels by regulation of osmogene activity. AE: ancillary element; APr: activating protein; ORE: osmotic response element; P: promoter. (After Gilles & Delpire 1997, modified).

(Gilles, Belkhir, Compere, Libioulle & Thiry 1995). Briefly summarizing the results obtained (Figures 9, 10 and 11), it can be shown that: (a) Synthesis of aldose reductase is induced within 24 h and sorbitol is the major osmolyte accumulated in cells submitted either abruptly or slowly to media made hyperosmotic (600 mOsm/l) by addition of NaCl or sorbitol. (b) There is no enzyme induction when proline is used to increase environmental osmolarity. Proline is in this case, the major osmolyte found in the cells. (c) Abrupt changes in osmolarity induce changes in the intracellular level of $K^+$ in the three media; large changes in $Na^+$ occur only in the NaCl medium. (d) Slow changes in osmolarity (0.3 mOsm/min) with NaCl or sorbitol induce no changes in $Na^+$ and changes in $K^+$ smaller than those recorded with proline under 'abrupt' conditions. (e) 'Abrupt' changes in osmolarity induce shrinkage and decrease in cell volume. No such modifications can be detected during 'slow' changes. (f) Aldose reductase and sorbitol remain high in cells long acclimated to their hyperosmotic medium. In such conditions, the cells have resumed volumes and $Na^+$-$K^+$ levels not significantly different from controls (Libioulle *et al.* 1996; Gilles *et al.* 1997 and unpublished).

These results indicate that: (a) Changes in external or internal osmolarity or in $Na^+$ level are not the signals primarily perceived by the cells. (b) Changes in volume do not seem to be implicated. (c) Changes in $K^+$ are not necessary for maintenance of high aldose reductase and sorbitol levels in acclimated cells. Changes in $K^+$ can be recorded in all our conditions of application of hyperosmotic shocks. A transitory change in $K^+$ in the early steps of acclimation might thus play some part in aldose reductase induction. Such a signal, if of any significant importance, cannot be the only one

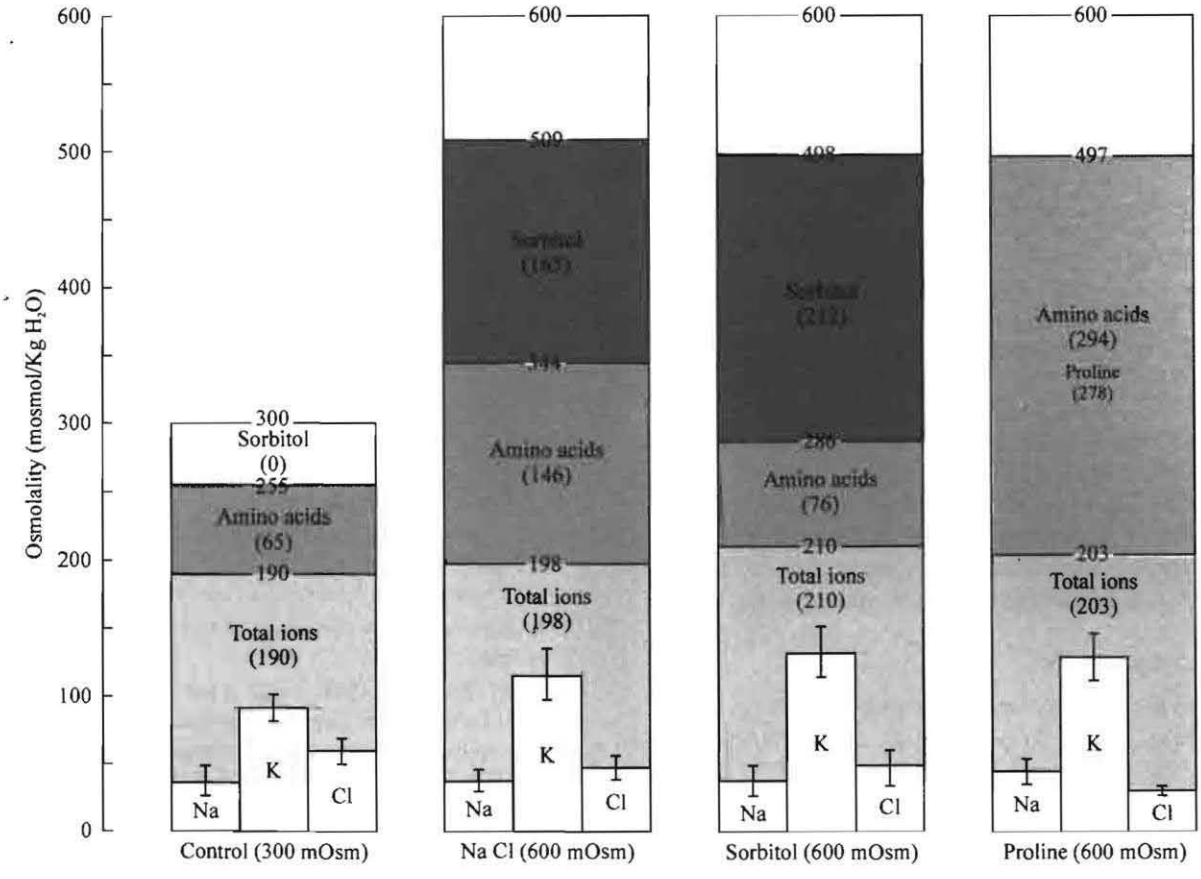



**Figure 9** Osmotic balance in L929 cells in control conditions (300 mOsm) or acclimated to media made hyperosmotic (600 mOsm) by addition of NaCl, sorbitol or proline (after Libioulle *et al.* 1996, modified).

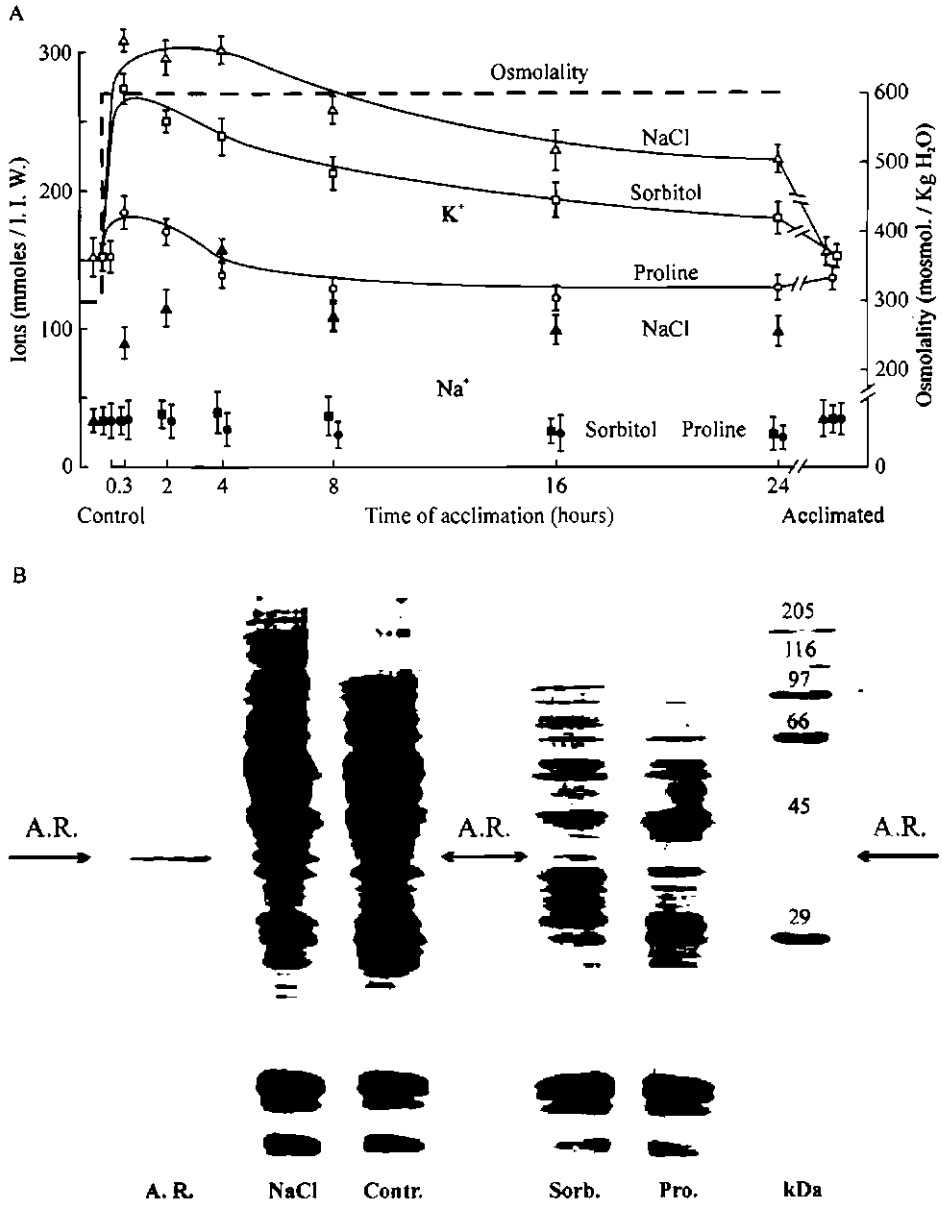



**Figure 10** Effect of application of an abrupt hyperosmotic shock (from 300 to 600 mOsm, broken line: ---) to L929 cultured cells on : (A) the evolution of the intracellular level of $Na^+$ (dark symbols) and $K^+$ (open symbols), (B) the 24 h induction of aldose reductase (A.R). The position of the enzyme after migration on an SDS gel is shown by the arrow. Media are made hyperosmotic by addition of NaCl (▲, Δ, sorbitol (■, □ or proline (●, ○). (Libioulle & Gilles, unpublished).

involved in triggering the enzyme synthesis. Other signalling or co-signalling system(s) are completely unknown. Possible candidates would be differential changes in membrane potential or in the configuration of some macromolecular component(s). These suggestions are by no means exclusive of others; any data in that field would be most welcome.

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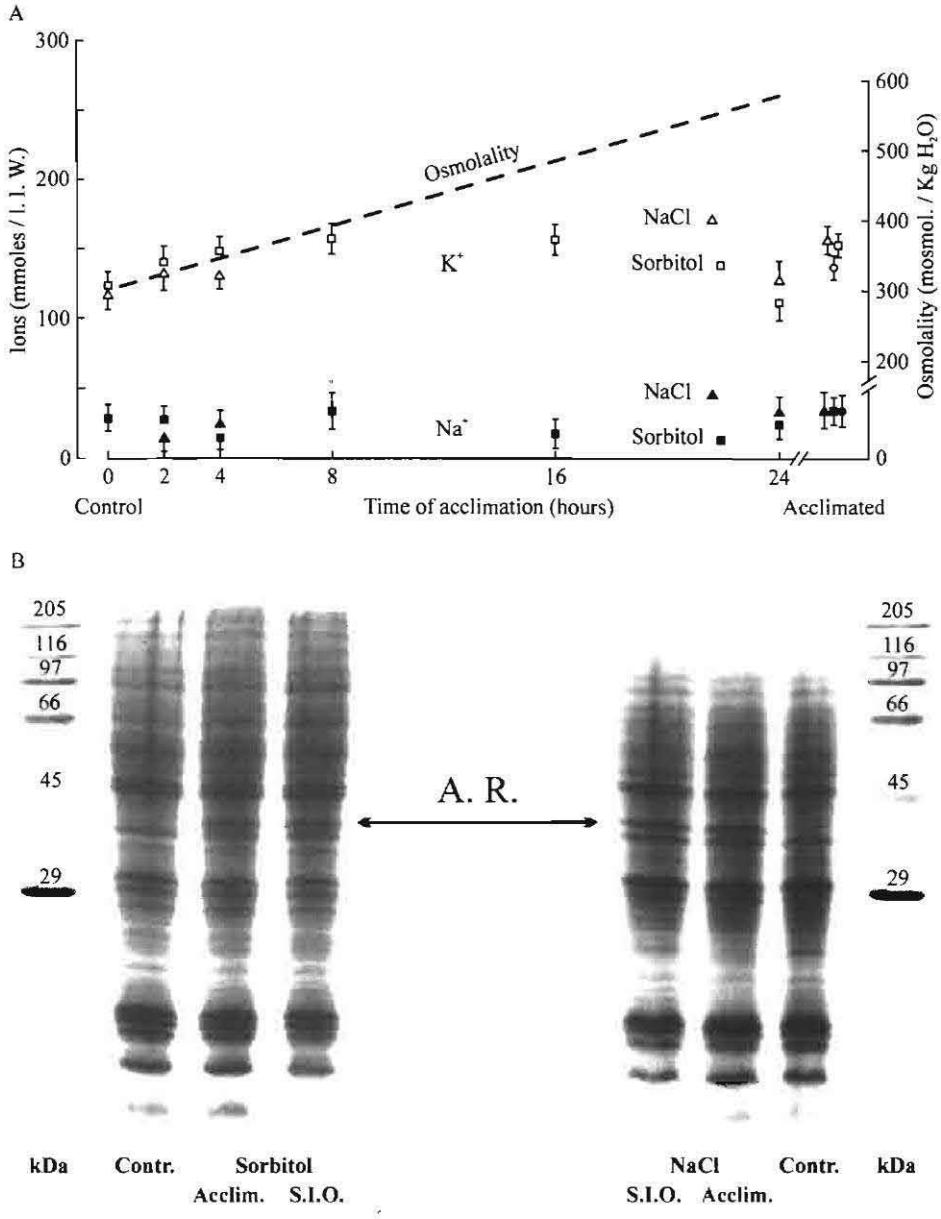


**Figure 11** Effect of application of a slow increase in osmolarity (from 300 to 600 mOsm – 0.3 mOsm/min, broken line: ---) to L929 cultured cells on: (A) the evolution of the intracellular level of $Na^+$ (dark symbols) and $K^+$ (open symbols, (B) the 24 h induction of aldose reductase (A.R). The position of the enzyme after migration on an SDS gel is shown by the arrow; S.I.O.: slow increase in osmolarity; Acclim: cells acclimated to a hyperosmotic medium. Media are made hyperosmotic by addition of NaCl (▲, Δ) or sorbitol (■, □) - (Libioulle & Gilles, unpublished).

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