Effects of hypo and hyperosmotic media on rabbit renal cortical slices[1]

R. Gilles, C. Duchêne and I. Lambert

Laboratory of Animal Physiology, University of Liège, 22, Quai Van Beneden, B-4020 Liège (Belgique), June 14, 1982

Summary. Rabbit kidney cortex slices behave as osmometers when withstanding either hyperosmotic shocks or hypo-osmotic shocks of amplitude up to $P_1/P_2 = 1.25$. For hypo-osmotic shocks of amplitude larger or equal to $P_1/P_2 = 1.5$ a volume regulation process occurs. Na^+ is the main osmotic effector implicated in volume control.

Cell volume regulation has been described for a variety of tissues and cell types of euryhaline poekilosmotic animals[2-5]. This phenomenon has considerable adaptive value since it enables the cells of these species to cope with the occasional but very large osmolality changes occurring at the blood level in different conditions of water availability in the environment. In homeosmotic animals, the problem of cell volume control in anisosmotic media has up to now received much less attention. In order to learn more about the capabilities of mammalian cells to cope with osmotic stresses, we undertook a study of volume regulation in rabbit kidney cortex slices submitted to anisosmotic media.

Material and methods. Adult white rabbits of about 2 kg were used for these experiments. They were stunned by a blow on the neck and their kidneys were removed, dissected and sliced as described elsewhere[6,7]. The cortical slices were immediately transferred to a preincubation isosmotic saline at 0.5 °C for a minimum of 45 min; the medium was renewed at least twice during that period. Incubation was achieved in glass vials containing about 10 slices in 10 ml of the adequate oxygenated saline. The saline was changed every 20 min. The preincubation and the control isosmotic salines had the following composition (mM): Na^+: 142.8; K^+: 5.3; Ca^{2+}: 1.5; Mg^{2+}: 1.2; Cl^-: 128.0; SO_4^{2-}: 1.2; acetate: 20.1; phosphate buffer 1.7 mM ph 7.4. The hypo- and hyperosmotic salines used had the same pH and ionic composition as the control saline except that their content in NaCl was varied to obtain the desired osmolality. In many experiments a hypo-osmotic saline with half the NaCl amount of control has been used; it is referred to in the text as NaCl/2 saline. The tissue water content was estimated by the fresh weight-dry weight technique. Na^+ and K^+ concentration measurements were performed by flame photometry after extraction of the tissue in 0.08N HNO_3.

Results and discussion. Figure 1 shows the effect of both a hypo-osmotic shock (NaCl/2) and a hyperosmotic shock (NaCl × 2) on the tissue volume (as determined from water content) of rabbit kidney cortex slices. The hyperosmotic medium induces immediate shrinkage of the tissue; no subsequent readjustment of the volume is observed, during our experiments. Application of a hypo-osmotic shock leads to a bisphasic response of the tissue; the initial swelling phase is followed by a slow decrease in cell volume. This is reminiscent of the classical biphasic response of cells of euryhaline poekilosmotic species to hypo-osmotic conditions that we have described previously[2,5]. The volume readjustment process shown by rabbit kidney cortex slices is much slower than the one described for many of the other tissues studied up to now; it is far from complete during the 150 min of our experiments. In figure 2 we have plotted the maximum change in volume recorded for osmotic shocks of different amplitudes. Kidney cortex slices behave as ideal osmometers and follow the van't Hoff relation in hyperosmotic situations as well as hypo-osmotic shocks of amplitude up to $P_1/P_2 = 1.25$. For hypo-osmotic shocks of amplitude equal to or larger than $P_1/P_2 = 1.5$, the maximum water content is lower than that expected on the basis of the van't Hoff equation. Some mechanism is at work in these conditions to limit the swelling of the tissue to levels lower than those expected when only considering water movements following a perfect osmometric behavior.

A so-called 'volume regulation' process has already been described on various mammalian kidney preparations (slices[6,7] isolated tubules[8-10]). It is, however, obvious that these studies have considered quite different phenomena under the same terminology. For Dellasega and Grantham[8], volume regulation is a very fast biphasic phenomenon taking place on about 2 min. In their system, the amplitude of the initial swelling is in agreement with the van't Hoff relation and the volume is 'regulated' in a very rapid shrinking phase taking place immediately after swelling. However, in a hypo-osmotic medium the osmolality of

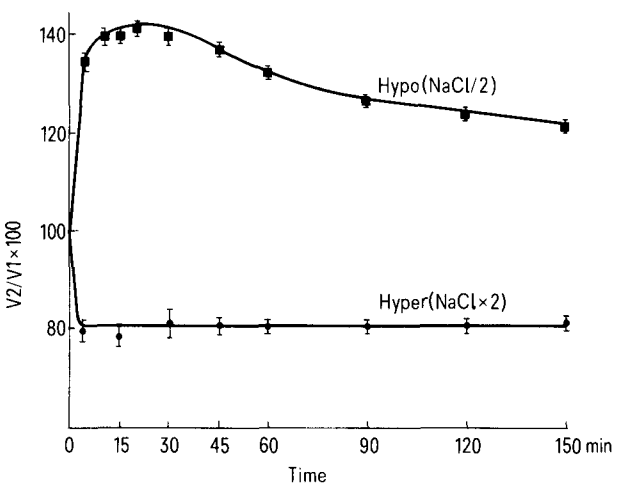

Figure 1. Effect of a hypo and a hyper-osmotic shock on the volume of rabbit kidney cortex slices. Tissue volume is expressed by its water content; V_1: water content in control condition, V_2: water content achieved in the anisosmotic medium.

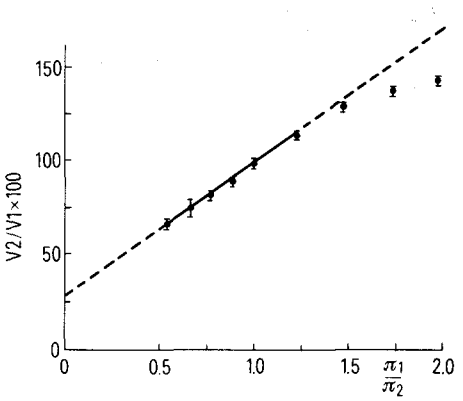

Figure 2. Effect of osmotic shocks of different amplitude on the maximum or minimum volume achieved by rabbit kidney cortex slices. P_1: osmolality of the control (isosmotic) saline; P_2: osmolality of the experimental (hyper or hypo-osmotic) saline.

which is half that of the control saline ($P_1/P_2=2$), the volume achieved after 'regulation' remains much larger than control. It is only for osmotic shocks of a much smaller amplitude ($P_1/P_2=1.28$) that a volume close to control is resumed in a slow phase of volume readjustment following the rapid biphasic answer of the tissue. The 'regulation' process was studied only for 30 min; such periods might have been too short to observe volume readjustment when the osmotic shock is significant. Paillard et al.[10] failed to show any volume readjustment in isolated tubules submitted to an important osmotic shock ($P_1/P_2=1.52$) during a 60-min period. The 'volume regulation' process described by these authors is in fact limited to a swelling limitation phase since the maximum volume reached by their preparation always remained smaller than that predicted on the basis of the van't Hoff equation. Further, the early rapid biphasic response leading to swelling limitation described by Dellasega and Grantham[8] cannot be observed in these experiments. Similarly the 'volume regulation' process described by Hughes and Macknight[6] on kidney slices submitted to hypo-osmotic conditions for 60 min consists essentially of a swelling limitation process with no early rapid biphasic phase. The results of these 2 groups of authors, as well as ours in the present study, thus fail to show the 'peak-shaped', biphasic nature of the swelling limitation process. This is most probably due to differences in the type of preparations used. The preparation of Dellasega and Grantham[8] allows a fast monitoring of the volume changes through camera recording while only indirect measurements are available with tissue slices or with the tubules preparation used by Paillard et al.[10]. If we now turn to the slow volume readjustment process, the time course of its evolution appears dependent on the amplitude of the osmotic shock applied and therefore on the amplitude of the initial swelling. On isolated tubules, volume readjustment is indeed completed within 15 min for a osmotic shock of amplitude $P_1/P_2=1.28$[8]. On the same preparation, as well as on kidney slices, no readjustment is observed on periods of 30 or 60 min for osmotic shocks of amplitudes equal to or larger than 1.52[6,8,10]. A significant volume decrease showing that a volume readjustment process is taking place can, however, be demonstrated on 150 min of experiment (Seel et al.[7], the present study).

The data of the different studies discussed up to now, when integrated in the light of our present results, lead thus to propose that the overall volume regulation process at work in kidney preparations implicates 2 different phases. Immediately after application of a hypo-osmotic shock, a very fast swelling limitation response of the tissue is taking place. In the preparations in which this phase can be 'visualized' such as in isolated tubules under camera recording, it appears as a peak-shaped, biphasic phenomenon during which volume rapidly decreases from its osmometrically predictable size to a smaller one. Swelling limitation could act, as we have proposed previously[4,5,11], to avoid a bursting of the cells due to a too large and too long lasting distention of the plasma membrane. In kidney, as in many other tissues, the swelling limitation process appears to be followed by a slow volume readjustment phase during which volume resumes values close to control ones.

As shown in figure 3, volume regulation is concomitant with a fast drop in the amount of Na^+. There is no significant change in the amount of K^+. These results confirm earlier findings by Hughes and Macknight[6] and Paillard et al.[10]. Similarly our results on the free amino acids patterns (not shown) demonstrate that there are only slight changes in the amount of these compounds in kidney slices during volume regulation. This makes it clear that neither these compounds nor K^+ can be considered important osmotic effectors in this tissue. This is at variance with the results obtained with most other tissues and cells types studied up to now. Indeed, in these preparations and depending on the species considered, changes either in K^+ alone (mammals, birds) or in K^+ and free amino acids (invertebrates, fish, Erlich cells) can account for most of the volume regulation (for recent reviews[4,5,11-13]).

Thus, contrary to what is found in most other tissues, control of the Na^+ intracellular content appears as the major process implicated in volume regulation in mammalian kidney. The mechanisms at work in the control of the Na^+ movements remain to be defined. Some results concerning this problem will be presented in a forthcoming paper[14].

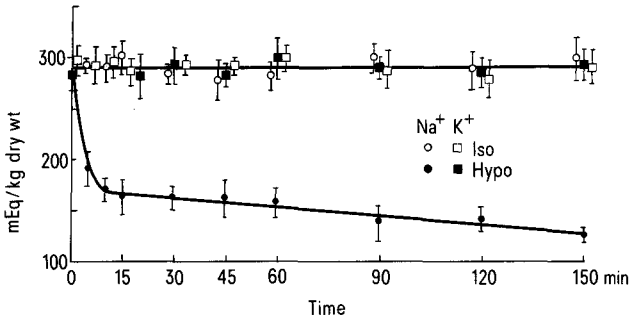

Figure 3. Effect of a hypo-osmotic shock on the Na^+ and K^+ content of rabbit kidney cortex slices. Results are given in mEq. per kg tissue dry weight.

1 This work has been aided by a grant 1.5.422.82F from the FNRS to R.G. We wish to thank Mr J.M. Theate for his skilful assistance.

2 Gilles, R., in: Marine ecology, vol.2, part 1, p.259. Ed. O. Kinne. Wiley Interscience, London/New York 1975.
3 Gilles, R., in: Osmotic and volume regulation - A. Benzon symposium XI, p.470. Eds C. Barker Jørgensen and E. Skadhauge. Munksgaard, Copenhagen 1978.
4 Gilles, R., in: Mechanisms of osmoregulation in animals, chp.4, p.111. Ed. R. Gilles. Wiley-Interscience, London/New York 1979.
5 Gilles, in: Animals and environmental fitness, vol.1, p.3. Ed. R. Gilles. Pergamon Press, Oxford/New York 1980.
6 Hughes, P.M., and Macknight, A.D.C., J. Physiol. *257* (1976) 137.
7 Seel, M., Rorive, G., Péqueux, A., and Gilles, R., Comp. Biochem. Physiol. *65A* (1980) 29.
8 Dellasega, M., and Grantham, J.J., Am. J. Physiol. *224* (1973) 1288.
9 Grantham, J.J., Lowe, C.M., Dellasega, M., and Cole, B.R., Am. J. Physiol. *232* (1977) F42.
10 Paillard, M., Leviel, F., and Gardin, J.P., Am. J. Physiol. *236* (1979) F226.
11 Rorive, G., and Gilles, R., in: Mechanisms of osmoregulation in animals, p.83. Ed. R. Gilles. Wiley Interscience, London/New York 1979.
12 Hoffmann, E.K., in: Animals and environmental fitness, p.43. Ed. R. Gilles. Pergamon Press. Oxford/New York 1980.
13 Kregenow, F.M., A. Rev. Physiol. *43* (1981) 493.
14 Gilles, R., Duchêne, C., and Lambert, I., Experientia *39* (1983) 600.

0014-4754/83/060579-02$1.50 + 0.20/0