Results. As soon as the calyx first appeared, i.e. at 9 days of incubation (d.i.), a slight and discontinuous reaction product was found along the surface membrane of this nerve terminal (fig. 1). Furthermore, as expected, endocellular AChE was observed in the cisternae of the rough endoplasmic reticulum, as proviously described[7]. At 12 d.i. the surface membrane was much more heavily labeled than at 9 d.i., although in a segment-like way (fig. 2, A). The intensity of labeling was quite variable and generally more marked at the 'neuronal' side of the calyx surface membrane than on the 'satellite' side, i.e. the side facing the satellite cell (fig. 2, A).

Quite often 'active sites' - which occur at points along the calyx itself - were markedly labelled as compared to adjacent membrane areas (fig. 2, B). At 15 d.i. - when the calyx is known to reach full maturation[8] - AChE reaction was intense and uniform, clearly labeling large surface areas typical of such nerve terminals at this developmental stage (fig. 3).

Control studies performed with BW284C51, an inhibitor of AChE, demonstrated no cytochemical reaction, indicating that the reaction product obtained in our experiments is indeed due to AChE activity. Finally under our experimental conditions we failed to observe diffusion of the reaction product.

Discussion. AChE activity localized at the surface of the ciliary neurons was first observed at 9 d.i., i.e. at the time of the first appearance of the calyciform synapse, the end product labeling both the neuronal and the satellite sides of the calyx surface membrane. Such a pattern of staining is not surprising, since AChE is known to occur all along the outer surface of the axonal membrane in cholinergic nerves[9]. During ontogenesis, however, differences emerge in AChE distribution between the neuronal side of the calyx and the satellite side, possibly due to a selective redistribution of AChE following the establishment of the axosomatic contact. Moreover, the fact that at 12 d.i. the neuronal side of the calyx appears more heavily labeled than the satellite one is in line with previous findings indicating that AChE is released from nerve terminals into the synaptic cleft, possibly serving as one source of postganglionic AChE[10]. In this context it must be emphasized that a heavier labeling of the neuronal side as compared with the satellite one still occurs in the adult CG[11]. From 12 to 15 d.i. the reaction product increases concomitantly with the ontogenetic enlargement of the synaptic area. There are in addition active sites which at these developmental stages appear to be markedly labeled by AChE reaction and are conceivably involved in ganglionic transmission. In this connection, however, it must be recalled that in the chick CG neurotransmission is fully developed at 7 d.i.[2] in spite of a minimal morphological differentiation of synapses at that developmental stage. Thus it is likely that AChE occurring in the newly formed calyx (9 d.i.) may already be involved in synaptic transmission.

The calyx-related distribution pattern of AChE on ciliary neurons appears to be closely linked to the establishment of the calyciform synapse, the calyx possibly acting as an inducing factor for a selective redistribution of AChE. During this period, i.e. from 9 to 15 d.i., functional synapses are forming between the ganglion and its target, i.e. the iris[12], so that retrograde iris-dependent influences on ganglionic AChE are already at work[1,13].

The overall distribution of ganglionic AChE is definitively stabilized only after the final pattern of neural connections has been established.

In conclusion AChE appears to be a suitable marker for synaptic maturation, and may also be useful for studying neuron-target cell interactions in the developing nervous system.

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Effect of ouabain on volume regulation of rabbit kidney cortex slices in hypo-osmotic media[1]

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Summary. The volume regulation process at work in rabbit kidney cortex slices submitted to hypo-osmotic media show both a swelling limitation and a volume readjustment phase. Swelling limitation is Na^+ dependent and is blocked by ouabain 10^{-3} M. There is, however, no need to implicate the activity of a ouabain sensitive Na^+/K^+ pump in this process.

It is now generally recognized that animal cells can regulate, at least partly, their volumes when subjected to hypo-osmotic conditions. During the past decade, this process has been shown to occur in a variety of tissues and cell lines from invertebrates and vertebrates (for recent reviews[2-10]).

Volume regulation is, in most cases, associated with a loss of intracellular K^+ and, especially in aquatic invertebrates, with a decrease in the level of free amino acids. In mammalian kidney, volume control appears to be essentially associated with a decrease in intracellular Na^+ [11-14].

Further, it has been shown that ouabain inhibits volume regulation in different kidney preparations[10,11]. This has been taken as evidence to state that the Na^+/K^+ ouabain sensitive pump is implicated in volume control in this tissue. This is at variance with what is found in most other tissues; the lack of effect of ouabain on volume readjustment has been reported many times[3,5,9]. In an attempt to clarify these problems, we have undertaken a study of volume regulation in rabbit kidney slices and of the effects of ouabain on this process.

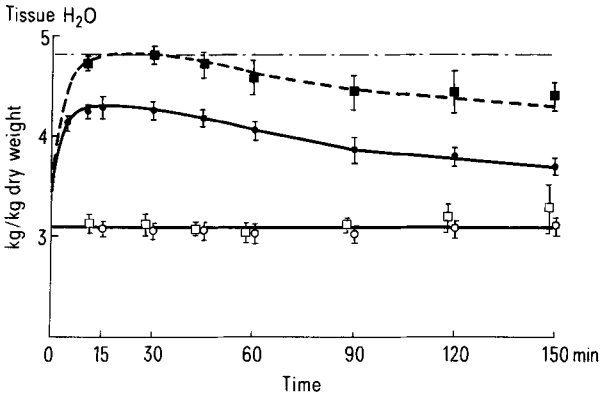

Figure 1. Effects of a hypo-osmotic shock and of ouabain (10^{-3} M) on the water content of rabbit kidney cortex slices. Results are expressed in kg · H_2O per kg tissue dry weight. Isosmotic conditions (○, □); hypo-osmotic conditions (●, ■); no ouabain (○, ●); ouabain added (□, ■). .- .-: Volume achieved considering an osmometric behavior of the tissue.

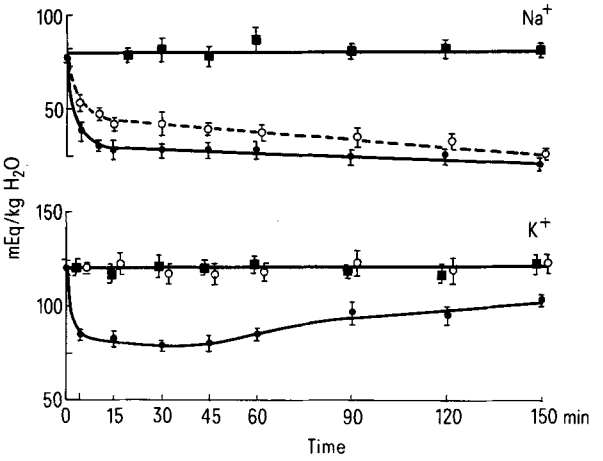

Figure 2. Effect of a hypo-osmotic shock on the intracellular level of Na^+ and K^+ in rabbit kidney cortex slices. Results are given in mEq. per l of intracellular water. ■, Isosmotic (control) conditions; ●, hypo-osmotic conditions; ○, results corrected for the changes in intracellular hydration.

Material and methods. Adult white rabbits of about 2 kg were used for these expeiments. They were stunned by a blow on the neck and their kidneys were removed, dissected, sliced and incubated as described previously[14]. The control isosmotic saline 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-osmotic saline had the same pH and ion composition as the control except that it had half its NaCl content. The extracellular space was estimated according to Roe et al.[15] using stable inulin. The preparations have always been incubated or preincubated for a minimum of 45 min in salines containing inulin 1% (W/V). 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 0.08 N HNO3.

Results and discussion. Volume regulation in rabbit kidney cortex slices submitted to hypo-osmotic conditions is shown in figure 1. As we have previously proposed when studying other tissues[4], this process can be dissociated into the 2 distinct phases of swelling limitation and volume readjustment. The maximum swelling observed is less than that expected on the basis of the van't Hoff equation when considering the tissue as an ideal osmometer, with an apparent osmotically inactive volume of 27%. This clearly shows that some mechanism is at work to limit the swelling of the cells at values lower than those which are expected. Swelling limitation is followed by a phase of volume readjustment during which the cells shrink slowly towards control volume. The volume readjustment process observed in rabbit kidney is, however, much slower than in many other tissues; it is far from complete during the 150 min of our experiments. Volume regulation of mammalian kidney tissue in hypo-osmotic conditions has already been described at least partly, in previous papers by Hughes and Macknight[11] and Paillard et al.[12]. Their observations were, however, limited to what is defined here as the swelling limitation process. Volume readjustment was overlooked since the experiments were only 60 min and since kinetics of volume control were not studied.

In most tissues and cell types, changes in the intracellular level of either K^+ alone (mammals, birds) or in K^+ and free amino acids (invertebrates, fish, Erlich cells) can account for most of the volume regulation[3,5,6,9,10]. Swelling

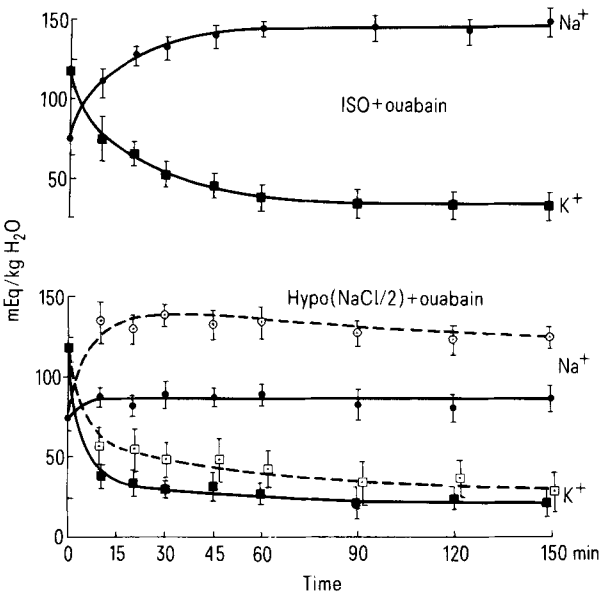

Figure 3. Effect of ouabain (10^{-3} M) on the Na^+ and K^+ intracellular content of rabbit kidney cortex slices incubated in isosmotic or hypo-osmotic conditions. ○, □, results corrected for the changes in intracellular hydration. Results are given in mEq. per l of intracellular water.

limitation in rabbit kidney, however, is concomitant with a fast drop in the intracellular amount of Na^+ (fig. 2). This confirms previous findings by Hughes and Macknight[11] and Paillard et al.[12]. Similarly, volume readjustment can be associated with a further slow decrease in Na^+. There is no significant change in the amount of K^+ during both phases. The changes in K^+ we observe disappear when the results are corrected for the variations in intracellular hydration. These results, as well as those of Hughes and Macknight[11] and of Paillard et al.[12] do not seem to agree with those of Grantham et al.[16] obtained on rabbit isolated kidney tubules. In their preparation, volume regulation seems to be related to a decrease in K^+ level and, to a lesser extend, in Na^+. As discussed elsewhere[12], at least part of this difference could be related to differences in the type of

preparation used. It should, however, be noticed that Grantham and coworkers use a preparation which seems to have a much lower Na^+ content. New data are awaited to shed light on this problem.

Our results further show that ouabain (10^{-3} M) blocks the swelling limitation process. Maximum swelling achieved in the presence of that compound coincides with that expected, considering a perfect osmometric behavior of the cells (fig. 1). This confirms earlier findings by Paillard et al.[12]. Our data also show that ouabain has no significant effect on the volume readjustment process. The lack of effect of ouabain on volume readjustment has already been reported in a variety of tissues and cell types[4,5,6,8,10]. In these cases however, volume readjustment can be related to a decrease in intracellular content of K^+ and/or amino acids due to an increase in plasma membrane permeability; the fact that ouabain has no effect is therefore not surprising. As far as kidney slices are concerned, our findings indicate that the Na^+ extrusion process concomitant with volume readjustment is not related to the activity of a Na^+/K^+, ouabain sensitive pump. Some other mechanism must therefore take an active part in the process. Further results should solve this problem.

Ouabain blocking of the swelling limitation phase has been considered as evidence that the activity of the ouabain sensitive Na^+/K^+ pump is involved in this process. However, our results show that in hypo-osmotic as well as in isosmotic conditions, ouabain induces a 1:1 exchange of Na^+ for K^+. In both cases, the increase in Na^+ level is indeed compensated by a similar decrease in K^+ and there is thus no net change in the total amount of intracellular osmotic effectors (fig. 3). This can easily account for the fact that ouabain has no effect on the tissue volume in isosmotic conditions. This can also account for the block of the swelling limitation process. Since there is no change in the total amount of osmotic effectors during that phase in the presence of ouabain, the cells behave as ideal osmometers. In this context, the decrease in Na^+ content associated with swelling limitation in the absence of ouabain can be explained simply by considering that 1. swelling limitation involves no change in the stoichiometry of the pump and leak system exchanging Na^+ for K^+ (1:1); 2. the intracellular level of Na^+ is determined by a readjustment of its ratio out/in at control level. In agreement with this is the fact that the ratios Na_o/Na_i remain in the same range in isosmotic conditions and during the swelling limitation phase (1.81 against 1.90 after 30 min of hypo-osmotic shock). It may be worth noticing that Na_o/Na_i ratio is much higher at the end of volume readjustment than in isosmotic conditions or during swelling limitation (3.11 against 1.81 or 1.90). This indicates that different processes are probably at work in the control of the Na^+ level in swelling limitation and in volume readjustment.

Studies on the mechanisms of Na^+ regulation implicated during that last phase are in progress in this laboratory.

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Cardiovascular compensatory and decompensatory responses in rats anesthetized with pentobarbital compared to chloralose-urethane[1]

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Summary. The data presented in these studies suggests that rats anesthetized with pentobarbital are better able to compensate for acute blood loss, but are less able to sustain the compensatory effort during hemorrhagic hypotension than rats anesthetized with chloralose-urethane. However, following reinfusion of shed blood the pentobarbital rats are better able to maintain their blood pressure.

One of the most important decisions facing biomedical scientists who use animals in their studies is whether or not the animals will require anesthesia; and if they do, which anesthetic or anesthetic regime is best suited for their particular study. In order to make a rational choice, one must be aware of the many side effects that accompany the use of anesthetics. For example, all anesthetics possess a CNS depressant action which places the animals in a state of unconsciousness and presumably renders them insensitive to painful stimuli. However, some anesthetics have been reported to have a greater depressant effect on discrete areas of the CNS than others, thus making them unsuitable for certain types of physiological investigation. For example Ruffy et al.[2] have shown recently that alpha-chloralose is better able to preserve the electrical properties of the heart subjected to reduced flow than secobarbital. In