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Biophysical properties of epithelial water channels

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Abstract

The biophysical models describing the structure of water pores or channels have evolved, during the last forty years, from a pure 'black box' approach to a molecular based proposal. The initial 'sieving pore' in which water and other molecules were moving together was replaced by a more restrictive model, where water is moving alone in a 'single file' mode. Aquaporins discovery and cloning [G.M. Preston, T.P. Carroll, W.B. Guggino, P. Agre, Science 256 (1992) 365] leaded to the 'hour-glass model' and other alternative proposals, combining information coming from molecular biology experiments and two dimensional crystallography. Concerning water transfers in epithelial barriers the problem is quite complex, because there are at least two alternative pathways: paracellular and transcellular and three different driving forces: hydrostatic pressure, osmotic pressure or 'transport coupled' movements. In the case of ADH-sensitive epithelia it is more or less accepted that regulated water channels (AQP2), that can be inserted in the apical membrane, coexist with basolateral resident water channels (AQP3). The mechanism underlying the so-called 'transport associated water transfer' is still controversial. From the classical standing gradient model to the ion–water co-transport, different hypothesis are under consideration. Coming back to hormonal regulations, other than the well-known regulation by neuro-hypophysis peptides, a steroid second messenger, progesterone, has been recently proposed [P. Ford, G. Amodeo, C. Capurro, C. Ibarra, R. Dorr, P. Ripoche, M. Parisi, Am. J. Physiol. 270 (1996) F880]. © 1997 Elsevier Science B.V.

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1. The water channel story: an overview

Thirty years ago we characterized the water permeability properties of the isolated muscle fiber [1]. Which were the measured parameters? The diffusion water permeability (P_d), the osmotic water permeability (P_{osm}), the activation energy (E_a) of both parameters and the reflection coefficient (σ) for a series of non-electrolytic, non-liposoluble molecules.

Which was the rational we were applying? Water was considered as moving across cylindrical, rectilinear pores. The Fick law and the Poiseuille law were respectively applied, when studying diffusion or filtration transfers. The non-electrolyte, non-liposoluble molecules were considered as moving together with water inside the pore, and their permeability properties were defined by a sieving mechanism. Associated to these ideas the 'solvent-drag' effect was described confirming the proposed mechanism (For a review on this points see Ref. [2]). On these basis a model showing 8 Å diameter pores was

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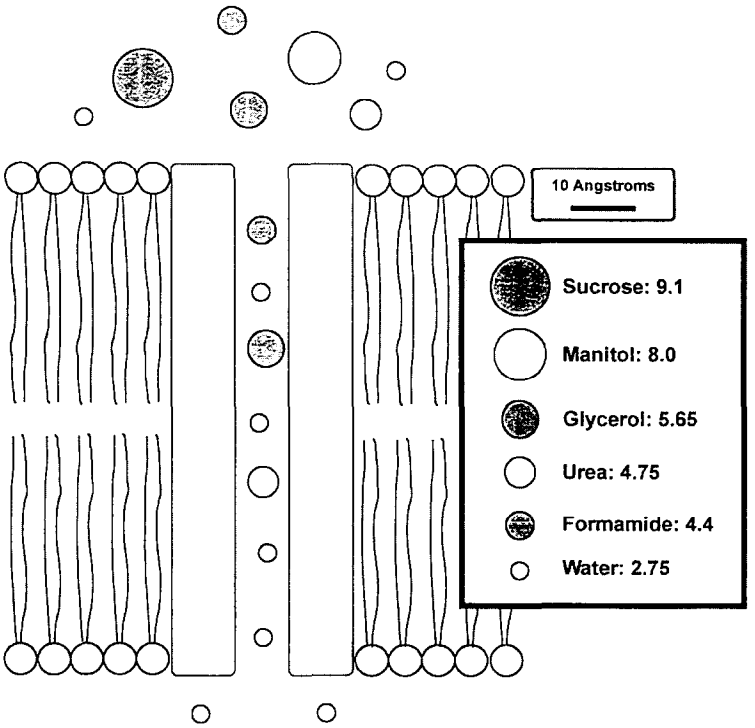

Fig. 1. Sieving pore model. Inspired by Goldstein and Solomon [5].

proposed for water transfer across animal cells [3–5] (Fig. 1). This model was also applied to the apical membrane in the case of epithelial barriers [6].

The introduction in the field of the unstirred layers concept [7] was a turning point. The 'pore model' was strongly challenged by experiments showing that unstirred layers, in series with the tested membranes, introduce strong artifacts leading to underestimated water permeability measurements. Simultaneously, the development of the artificial lipid bilayer technique gave additional information indicating that the bilayer water permeability could be rather high [8,9]. At this moment an alternative model was proposed: water would be moving across the cell membranes by a 'partition–diffusion' mechanism [6,10] (Fig. 2).

The use of 'artificial channels', that could be incorporated to the lipid bilayer, again modified the current model on water transfer across biological membranes. In this aspect, the Finkelstein experiments were crucial, reintroducing the idea of a specific pathway for water [11]. He showed that in pure lipid bilayers, after adequate corrections for unstirred

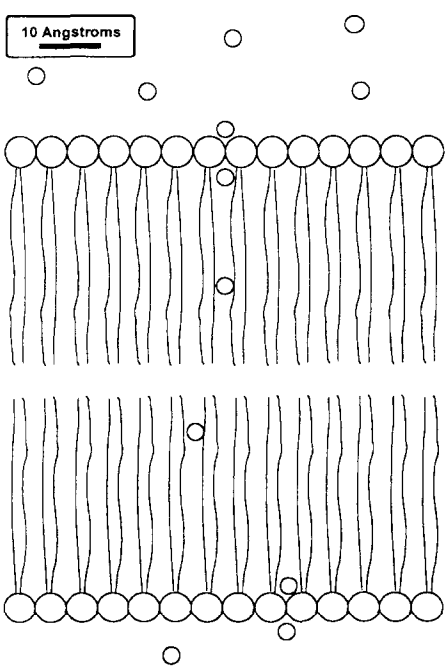

Fig. 2. Difussion–partition model. See Finkelstein and Cass [2].

layers effects, P_{osm} equals P_d, indicating a partition–diffusion mechanism. Nevertheless, when a channel forming molecule is added, it can be demonstrated that

$\Delta P_{osm}/\Delta P_d = n$

n being a number that characterizes the inserted channel. This was the birth of the 'single file hypothesis' [12,13]. An interesting experiment was to observe the evolution in P_{osm} and P_d as a function of the number of gramicidin channels inserted in a lipid bilayer [14,15]. At zero channel, $\Delta P_{osm}/\Delta P_d = 1$ but as soon as gramicidin channels were incorporated, $\Delta P_{osm}/\Delta P_d = 5$. This ratio was interpreted as the number of water molecules that accommodates inside the structure in a 'single file' mode.

These ideas were applied by us to the antidiuretic hormone (ADH) controlled water channel. The simultaneous measurement of P_{osm} and P_d allowed us to postulate a 'quanta' mechanism for water channel incorporation after ADH stimulus [16]. In a second step, we proposed the 'single file hypothesis' for the ADH controlled water channels in epithelia [17].

Which were the experimental supports for the new model? We reported a $\Delta P_{osm}/\Delta P_d$ ratio > 10 for the antidiuretic hormone (ADH) controlled water channel. This figure was confirmed by Levine et al. [18]. Additional support came from freeze fracture studies showing the appearance, in the ADH target membranes, of intra-membrane particle aggregates, whose number was proportional to the increase in water permeability [19,20]. Finally we demonstrated that manipulation of the intracellular pH can trigger an 'on–off' mechanism opening and closing water channels [21,22]. The water channel was now visualized as a narrow and rather long structure (Fig. 3).

At that moment an additional observation related to the properties of water channels was described: its osmotic permeability can be blocked by mercurial agents [23–25]. Even when the action of mercurial compounds is rather non-specific, it became an important tool when studying water permeability.

In summary, the incorporation of water channels to a cell membrane appeared as supported by the following experimental observations: (1) an enhancement of water permeability; (2) a $\Delta P_{osm}/\Delta P_d$ ratio > 10; (3) the development of sensitivity to mercurial compounds; (4) a reduction in the E_a for the water transfer.

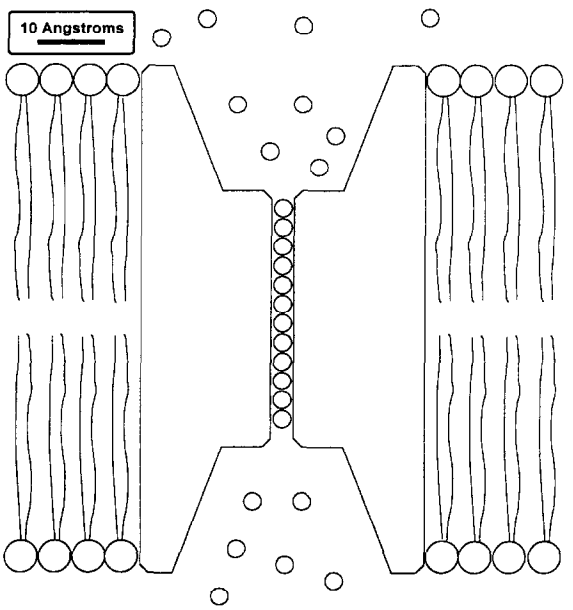

Fig. 3. Single file model. Inspired by Parisi and Bourguet [17] and Levine et al. [18].

At that moment we proposed an apparently audacious idea: the water channels are present as a widespread structure in the animal cells [26].

In 1992 Preston et al. reported the appearance of water channels in Xenopus oocytes expressing a red cell protein, that they called CHIP28 [27]. This leaded to a new conception of the problem, that can be summarized in the following experimental results: When the water permeability of *Xenopus laevis* oocytes is studied, it can be observed that $P_{osm} = P_d$. It is concluded, applying the previously developed rationalism, that they do not have water channels and that water would be moving by the partition–diffusion mechanism across the lipid bilayer. If now the total mRNA obtained from a tissue where water channels are expressed is injected into the Xenopus oocytes, an increase in P_{osm} and P_d is observed, and $\Delta P_{osm}/\Delta P_d > 10$.

Furthermore, a sensitivity to mercurial agents is developed, and the activation energy is reduced [28]. This was the first step to the cloning of water channels. Aquaporins were born. Typical expression and cloning experiments as those reported by Preston et al. [27] were extended to the kidney by Zhang et al. They reported the cloning, functional analysis and

cell localization of a kidney proximal tubule water transporter homologous to CHIP28 [29].

At the present moment an impressive series of water channels have been cloned. They have important structural analogies and the name aquaporin (AQP) was proposed for water permeable members of this protein family [30]. CHIP-28 became AQP1. The experience has demonstrated that our 'audacious' idea was a rather conservative one. Water channels have been described in bacteria, higher plants, insects, amphibian, mammals, including humans (see Refs. [31,32]). We are not going to develop the 'molecular biology' story. We will remain in the biophysical approach. From this point of view, and on the basis of two-dimensional crystallography at 3.5 Å resolution [33–37], the three-dimensional structure presented in Fig. 4 was proposed. Fig. 4a shows an upper view of the proposed model. Each six-barrel unit is an aquaporin. As it can be observed, a tetrameric structure defines a vestibule, darker zone, where four 'small holes' are the sieving structure for the water transfer. If we made a transversal section following the dashed line, the figure presented in Fig. 4b is obtained. Now a different gray intensity indicates two different aquaporin molecules. Of course this is just a model and important work is still necessary to define the water channel molecular structure. Another important task will be to develop a biophysical frame describing the water transfer across such a structure. The study of the interactions between water and channels, in artificial systems, can still give significant information on the biophysical properties of water channels [38].

2. The driving forces moving water across epithelial barriers

We will now go to the analysis of water transfers across epithelial barriers. Three different situations are classically described: (1) A net water movement (J_w) is driven by a transepithelial gradient of hydrostatic pressure (ΔP); (2) the observed J_w is generated by a transepithelial osmotic gradient ($\Delta \Pi$); and (3) the J_w is associated to ionic transport and can be observed in the absence of any transepithelial $\Delta \Pi$ or ΔP.

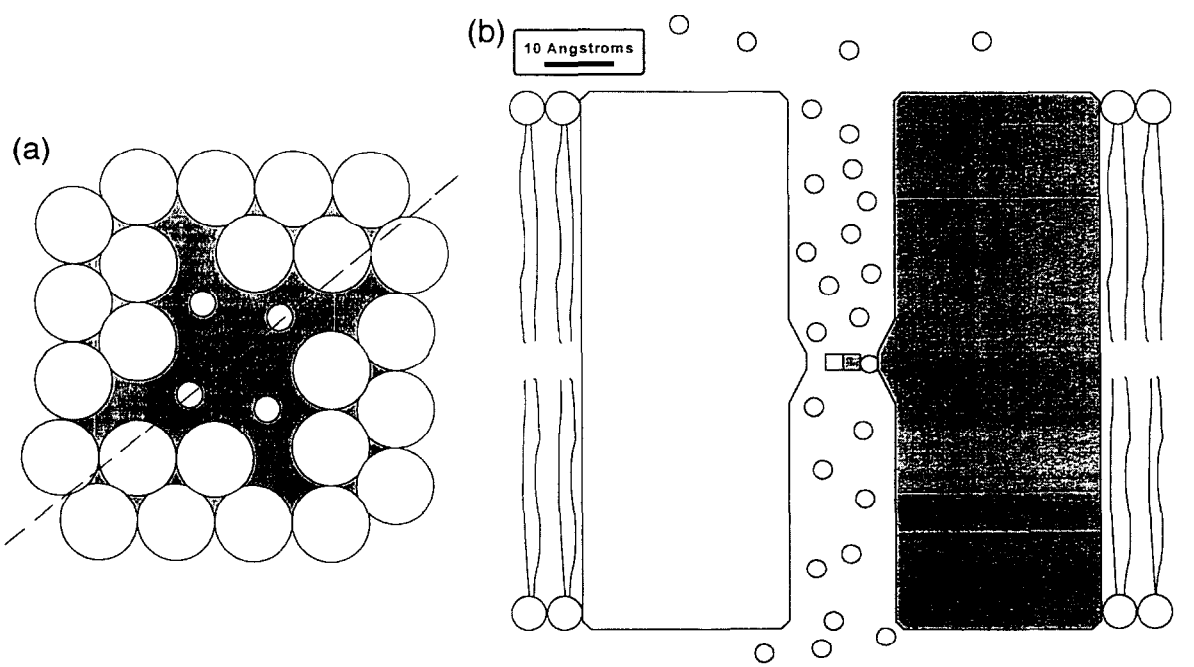

Fig. 4. Hour glass model. Inspired by Jung et al. [33], Walz et al. [34,36], Mitra et al. [35] and Jap and Li [37]. (a) Upper view of the model. (b) Transversal view of the model.

The hydraulic permeability (P_f, cm/s) is classically described as the amount of water moving per unit of time and surface in the presence of an osmotic or hydrostatic gradient. We will differentiate here, according to the type of gradient, the hydrostatic permeability (P_{hydr}) from the osmotic permeability (P_{osm}).

2.1. Water transfers induced by a ΔP transepithelial gradient

It is generally accepted that this type of net water movements are paracellular. The typical case is plasma filtration in the renal glomerulus and, to a lesser extend, in the capillary wall (and other endothelial barriers). In this filtration process water and small solutes (charged or not charged) would move together. The physiological role of transepithelial hydrostatic gradients in other epithelia has been widely discussed [39–43], in the frame of a main concept: epithelial barriers are tight or leaky, on the basis of its transepithelial electrical resistance. Nevertheless, 'electrically leaky' does not necessary means 'water leaky' and conductance and water permeability can be dissociated in certain epithelial barriers. In the rabbit rectum for instance, serosal hypertonicity increases the mannitol permeability together with a decrease in transepithelial conductance and no change in P_{hydr} [44].

The tight-junction structure has a central role in the control of the paracellular pathway [45]. Concerning paracellular water permeability we can mention the amphibian urinary bladder [46] and the mammalian rectum [44] as two structures where P_{hydr} is quite low. In both cases the tight-junction structure present several strands in freeze-fracture studies. It has been also observed that in the small intestine [43], the human amnion [47] and in Caco-2 cells [48] the cellular pH can control a relatively elevated paracellular water permeability.

Several components of the tight junction have been identified in recent years and characteristics of these proteins suggest how the structure might be organized and regulated [49]. However, no definitive structure has been described in terms of 'paracellular water channels'.

2.2. Water transfers induced by a $\Delta\Pi$ transepithelial gradient

Both paracellular and transcellular water movements, induced by transepithelial osmotic gradients, have been described. Concerning the paracellular route again the classical model is the capillary wall. Plasma proteins, that do not permeate the cellular junction, create the transepithelial $\Delta\Pi$ that would move water through this pathway from the interstitial space into the blood vessels. Nevertheless, the discovery of water channels in certain capillary walls [50] challenged the classical view. It is now proposed that in those endothelial barriers in which an important osmotic J_w is observed, the movement can be transcellular.

The transcellular route is generally accepted in the case of antidiuretic hormone (ADH) target tissues. These epithelial barriers (collecting duct in mammals, amphibian urinary bladder) present a relatively elevated P_{osm} at the basolateral membrane while the apical membrane can switch from a low to a high P_{osm}. Even before the cloning of the ADH regulated aquaporin (AQP2, [51–53]) and on the basis of biophysical [13] and freeze-fracture [54] studies it was proposed that, under ADH, water channels stocked in intracellular vesicles are transferred to the apical membrane [55]. In the case of the basolateral membrane, the water channels would be resident ones, as it was demonstrated in molecular biology experiments (AQP3 in Refs. [56–58].

In summary, we can accept three different situations concerning a J_w driven by a transepithelial $\Delta\Pi$: (1) a paracellular route (many capillary walls, small intestine); (2) a transcellular route in which both the apical and the basolateral membranes contain water channels ('high osmotic permeable' endothelial barriers); and (3) a transcellular route, with resident channels in the basolateral membrane and regulated channels in the apical border (ADH sensitive tissues, perhaps distal colon mucosa).

2.3. Transport associated water movements

This has been and still is a controversial subject. Water and salt are isotonically reabsorbed in different tissues: the gall bladder, the small intestine, the

proximal renal tubule. This absorptive flow can be observed in the absence of any transepithelial ΔP or $\Delta \Pi$. The standing gradient theory [59] remains the central reference in the field. According to this model a salt (NaCl for example) accumulates in the intercellular space, as a consequence of active sodium transport in the basolateral membrane. This space becomes then hypertonic and water will enter in it through the tight junction, inducing dilation and creating a hydrostatic gradient that will drive the fluid into the serosal side.

The standing gradient model has been widely discussed [60]. We will not review here the arguments concerning salt accumulation in the intercellular space, but water transfers via the paracellular route became rapidly a controversial point. It was proposed that in the proximal renal tubule water would move, at least partially, transcellularly [61]. The cloning and expression of a water channel, located in the tubular brush border [29] gave strong support to the transcellular model, at least in this tissue. Nevertheless, this situation puts a new problem that it is not yet solved: where is the local osmotic gradient created that couples ion and water movements? Furthermore, water can be transported against an osmotic gradient and a co-transport of K^+, Cl^- and water in the Necturus choroid plexus as it has been proposed in Ref. [62].

Hypertonic re-absorption also plays an important physiological role. The concentration mechanism in the renal medulla can be explained by an important salt re-absorption in the ascending limb of Henle that is not associated to water re-absorption. As a consequence, the urine concentration decreases along the distal tubule while the interstitium concentration is going up. In parallel to this, the descending limb is quite water permeable, allowing a rapid osmotic equilibrium between the descending fluid and the interstitium. Molecular biology experiments corroborate this view: water channels are expressed in the descending but not in the ascending limb of Henle [63]. Hypertonic re-absorption is also observed in the rabbit descending colon [64] and in the salivary glands [65].

Water and solute coupling is still less understood in secretor processes [66]. At least in the case of Caco-2 cells cultured on permeable supports, hypotonic secretion has been reported [48].

3. Regulation of water channels in epithelial barriers. The progesterone action

Water channels are classified into two main types: resident water channels, 'permanently' present in the considered membrane, and 'regulated water channels' in which the number in the target membrane is controlled by antidiuretic hormone. Furthermore, as previously mentioned, they can be open or close according to the intracellular pH [21,22] and medium hypertonicity can also triggers an 'ADH-like' hydrosmotic response [67]. It has been recently proposed that hypertonicity is regulating AQP2 at the corresponding gene level [68]. We have recently demonstrated that progesterone can be a second hormonal regulator [69]. Because this is a quite novel proposal, we will described this in a more detailed form.

Before the analysis of the regulation of water permeability in epithelial barriers, let us describe these mechanisms in the amphibian oocytes, a general expression model. As previously mentioned, Xenopus oocytes do not have water channels. We studied the water permeability properties of the oocytes from *Bufo arenarum*, an amphibian species living in the Buenos Aires area. When comparing P_{osm} in oocytes from Bufo and Xenopus it was observed that in Bufo the water permeability is higher, together with the appearance of a reversible sensitivity to mercurial agents. Furthermore, when we compared the activation energy for water transfer in both species, lower values were observed in Bufo. Furthermore, the water permeability is sensitive to acidification of the intracellular pH in Bufo but not in Xenopus oocytes and subtracting Xenopus from Bufo permeability values, it was possible to calculate a $\Delta P_{osm}/\Delta P_d$ ratio that is higher than 10. All conditions were fulfilled to propose that Bufo oocytes contain water channels that are not present in Xenopus oocytes [70]. This is strongly supported by an additional experiment: injection of the total mRNA from Bufo in Xenopus oocytes induced the expression of an increase in P_{osm} that was sensitive to mercurial agents [69].

Bufo arenarum has a rather terrestrial habitat, quite different from Xenopus, that spends its life in water. On the other hand, it is known that progesterone induces important changes in amphibian oocytes during their arrival to maturity [71]. We

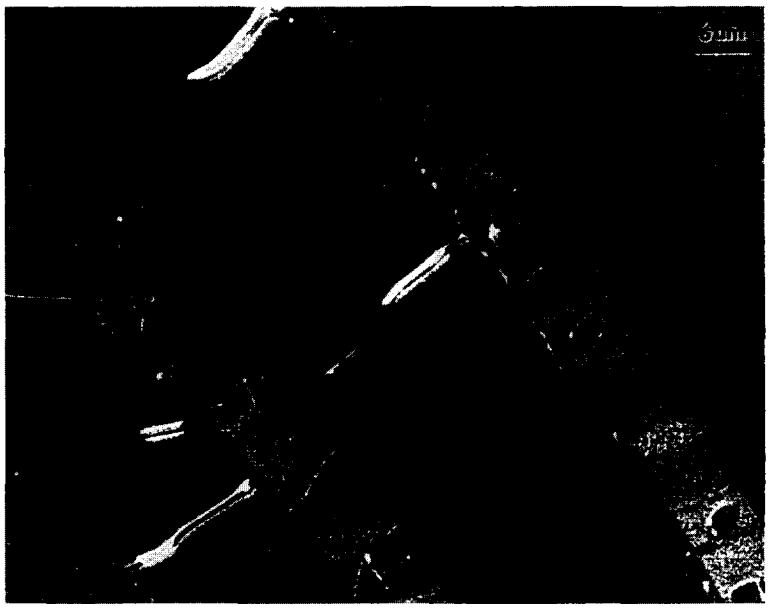

Fig. 5. Caco-2 cells forming an epithelial barrier on a permeable support (Nucleopore filter, 3 μm pore diameter).

tested the effects of this hormone on the water permeability of Bufo oocytes. They were treated with progesterone immediately before or 18 h before the permeability measurement. Progesterone induced a significant inhibition of P_{osm} only after a 18 h lag. Furthermore, only the mercurial sensitive fraction of the measured permeability was inhibited by the hormone [69]. We can conclude that the presence of water channels in the membrane of Bufo oocytes is depending on the expression of the corresponding RNA messenger and controlled by progesterone at the transcription level.

Let us switch to epithelial barriers. The toad urinary bladder has been widely used as a model system to study the action of ADH [46,72] and the sequence and functional expression of an amphibian aquaporin (FA-CHIP) has been obtained from frog urinary bladder tissues [73,74]. When the hydrosmotic response to ADH in *Bufo Arenarum* urinary bladders was twice tested with an 18 h interval, it was observed that the magnitude of the response was similar, even though slightly slower the second time. Nevertheless, if after the first stimulus the bladders were exposed to progesterone during 30 min, an inhibitory effect was detected, 18 h later [69]. It is known that when the mRNA from toad urinary bladder cells is injected into Xenopus oocytes, an increase in water permeability is observed [75]. This increase is blocked if the bladders are pre-treated with progesterone 18 h before the mRNA extraction [69]. We can conclude that the progesterone-regulated water channel present in the ovarian oocyte of *Bufo arenarum* shares its progesterone sensitivity with the ADH-regulated water channel, present in the urinary bladder of this species.

4. Towards the reconstitution of aquaporin-containing epithelial barriers

To study the water permeability properties of natural epithelial barriers is not easy because the existence of multiple cell types and complex supracellular structures (crypts, villae, etc.) as well as subepithelial components (muscular, vascular and conjunctive tissues). Because of this situation we have developed an experimental system in which P_{osm} and P_d can be simultaneously and continuously measured in epithelial layers formed by cloned cell lines cultivated on a permeable support [48,76]. Fig. 5 shows a transversal view of Caco-2 cells forming a cell monolayer at confluence on a 3 μm-pore Nucleopore filter.

We have studied the water permeability properties of a series of cell lines in culture: Caco-2, T-84, LLCPK, MDCK. We analyzed the effects of changes in medium pH on the measured parameters [48]. We also tested different osmotic and hydrostatic gradients [44,48]. From these biophysical studies as well as from inmuno-localization studies it was concluded that these cell lines do not express water channels. Because of this situation they became excellent candidates for transfection studies. Cloned aquaporins have been transfected into epithelial cell lines [77]. The culture of these transfected epithelial cells on a permeable support gives a quite useful tool to characterize its permeability properties in reconstruction experiments. This is the subject of our present work.

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