Aqueous humour (AH) is an important intraocular fluid responsible for the supply of nutrients to and removal of metabolic wastes from the avascular tissues of the eye. It is also indispensable for the maintenance of the optical properties of the eye. The fluid dynamics of AH are frequently associated with the potentially blinding disease called glaucoma. Pharmacological treatment of glaucoma generally aims to lower the intraocular pressure by reducing AH formation. However, the mechanism underlying the formation of AH is still not well understood. Understanding the mechanism of AH formation and its regulation is paramount to develop rational and target specific drugs for the treatment of glaucoma. It is now generally believed that AH is formed mostly by active transport of ions and solutes across the ciliary epithelium. Many studies have been carried out in the past half a century to understand these transport processes. In the past several years, new information has emerged and a comprehensive review of these new developments is necessary. This review covers the ion transports in the ciliary epithelium, including the possible roles of sodium, chloride and bicarbonate ions as the driving forces. It also examines the current ionic models for AH formation and its regulation from a cellular transport perspective.

Key words: active secretion, aqueous humour, ciliary epithelium, ion transport

Aqueous humour (AH) is a transparent fluid contained in the anterior and posterior chambers of the eye and is formed by the ciliary epithelium (CE) of the ciliary processes projecting from the ciliary body. It is secreted into the posterior chamber of the eye by the CE, a double layer of cells located on the surface of the ciliary processes. It then circulates through the pupil into the anterior chamber. A large portion of AH leaves the eye through the trabecular meshwork into Schlemm’s canal and the episcleral venous system. The remainder escapes via the uveoscleral route by simple percolation though the interstitial tissue spaces of the ciliary muscle into the suprachoroid and out through the sclera. In human, the total volume of aqueous humour is approximately 250 µl and the average rate of its formation is about 2.75 µl/min.

The circulation of AH to the anterior segment of the eye serves a number of important functions:

1. it supplies nutrients and oxygen to and removes metabolic wastes from the anterior intraocular tissues such as cornea, crystalline lens and trabecular meshwork
2. it inflates the globe and creates an intraocular pressure (IOP) for normal optical functioning of the eye
3. it transports ascorbic acid (AA) into the anterior segment where it serves as an antioxidative agent by scavenging free radicals
4. it facilitates the local immune responses during inflammation and infection. The chemical composition of AH differs from the blood plasma. The most striking difference between the two fluids is the protein and AA concentrations. The protein concentration in AH ranges from 0.05 to 0.15 g/l, while its concentration in blood plasma ranges from 60 to 70 g/l. It
Aqueous humour formation

is virtually free of proteins; this is essential for maintaining optical transparency.6,7 Although the protein concentration in the AH is very low, all the plasma proteins are present in the AH. This suggests that AH proteins are primarily derived from the blood plasma rather than being synthesized locally.1,4 Furthermore, a high concentration of AA is present in aqueous humour of different animal species.8,9 In rabbit, for example, the concentration of AA in the anterior and posterior chambers is higher than that in the blood plasma by approximately 50 to 70 fold.1,10 This suggests that there must be a mechanism to concentrate AA in the eye. The AA in AH may distribute to the surrounding intraocular tissues to protect the eye from the photo-oxidative damage.11,12

It has been demonstrated that AH is not a simple filtrate of the blood plasma. The concentration of various ions (for example, HCO₃⁻ and Cl⁻) in AH differs significantly from that of blood plasma and the difference is larger than expected from the Gibbs-Donnan equilibrium. In addition, the distribution of these ions varies among different species (Table 1). In rabbit and guinea pig, for example, there is an excess of HCO₃⁻ and a deficit of Cl⁻ in the AH compared to blood plasma. In contrast, there is an excess of Cl⁻ and a deficit of HCO₃⁻ in the AH of horse and human.5 This difference is reflected in the pH of the AH, which is largely determined by the HCO₃⁻ concentration; in rabbit, the pH is alkaline, while in human, it is acidic relative to blood pH.4

Because the AH is of great importance to the normal functioning of the eye, an optimal aqueous flow level must be maintained. The IOP is determined by the dynamic balance between AH formation (inflow) and drainage from the eye (outflow) through the trabecular meshwork and the uveal scleral pathways. Primary open angle glaucoma is the name given to a group of conditions in which retinal ganglion cells die due, at least in part, to an increase in IOP. The elevated IOP causes optic atrophy resulting in irreversible vision loss by unknown mechanism. It has been estimated that about one to two per cent of the population in the world suffer from glaucoma-related vision loss.13 Although the aetiology for IOP elevation is believed to stem from reduced outflow facility, the pharmacological reduction of IOP by suppressing AH formation remains the mainstay of glaucoma treatment. However, it is surprising that the mechanisms of AH formation and its regulation are poorly understood. It is the purpose of this paper to review these processes.

AQUOUS HUMOUR FORMATION

Aqueous humour formation is a complex process. Basically, it can be subdivided into four stages.

1. There must be blood flow to the vascular bed of the ciliary processes.
2. An ultrafiltrate is passed through the fenestrated capillaries of the ciliary processes, which have high protein per-

<table>
<thead>
<tr>
<th>Substance</th>
<th>AH*</th>
<th>BP*</th>
<th>AH/BP ratio</th>
<th>ANT*</th>
<th>BP*</th>
<th>ANT/BP ratio</th>
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<td>Sodium ions</td>
<td>143.5</td>
<td>151.5</td>
<td>0.95</td>
<td>152</td>
<td>148</td>
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<tr>
<td>Potassium ions</td>
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<td>3.9</td>
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<tr>
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<td>107</td>
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<td>Ascorbate</td>
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<td>Hydrogen ions (pH)</td>
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<td>1.03</td>
<td>7.21</td>
<td>7.4</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 1. Chemical compositions of aqueous humour (AH) or anterior aqueous (ANT) and blood plasma (BP) of human* and rabbit‡

* Concentrations are expressed in micromoles per millilitre
† Data from studies of monkey
AH/ BP ratio, aqueous humour to blood plasma ratio
ANT/ BP ratio, anterior aqueous to blood plasma ratio

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3. Active transport, which is an energy-consuming process involving the movement of solutes across the CE against their concentration gradients. Previously, it was thought that ultrafiltration contributed about 80 per cent to aqueous humour secretion. However, Bill reported that the difference in the hydrostatic pressure was much less than the difference in the oncotic pressure between ciliary stroma and AH. This differential pressure across the CE tends to move water from the posterior chamber into the ciliary processes (absorption) rather than facilitate ultrafiltration into the posterior chamber. Several subsequent studies have showed that the formation of AH can be suppressed by a variety of metabolic inhibitors, anoxia and hypothermia, suggesting a role for active transport as a major mechanism for aqueous secretion. In addition, Burstein and colleagues have shown that sustained fluid secretion occurs against a hydrostatic gradient. In arterially-perfused bovine eye, Wilson, Shahidullah and Millar found that increasing the perfusion rate raised the perfusion pressure but had little effect on IOP. These results suggest that the formation of AH involves active transport rather than ultrafiltration. It is estimated that the relative contribution of ultrafiltration to AH formation is about 20 to 30 per cent.

CILIARY EPITHELIUM

The CE is a double layer of cells covering the surface of the ciliary body. The outer layer which abuts to the aqueous side or vitreous body is called the non-pigmented epithelium (NPE), whereas the inner layer is heavily pigmented and is called the pigmented epithelium (PE). The two layers face each other in an apex-to-apex fashion, which is a result of the embryological invagination of the optic cup.

The CE is widely accepted as the site of AH formation. A regional difference in the distribution of ion transporters has been demonstrated in CE and this may have functional and physiological significance. The ion transporters usually concerned with epithelial secretion were found to be more numerous at the anterior region (pars plicata) than the posterior region (pars plana) of the ciliary body, suggesting that the pars plicata is the primary site of AH formation.

Pigmented epithelium
The PE of the ciliary body represents the forward continuation of the retinal pigment epithelium. The infoldings of the basal surface of the PE layer provide a large surface area facing towards the stroma of the ciliary processes, thereby facilitating solute uptake from the blood plasma. In addition, there are numerous interdigitations and gap junctions at the lateral borders of the cells. The cytoplasm of PE cells contains many pigment granules but fewer mitochondria than in non-pigmented epithelial cells.

Non-pigmented epithelium
The inner NPE is the forward continuation of the neural layer of the retina. Like the PE cells, the NPE cells also possess numerous basal infoldings and interdigitations at the lateral surface. This acts to increase the surface area, which is a characteristic feature of secretory epithelia concerned with fluid transport. When compared with the PE cells, the NPE cells have more mitochondria and rough endoplasmic reticulum. Moreover, the NPE has higher Na+K+-ATPase and adenylyl cyclase activity, indicating that the NPE cells are more metabolically active. It has been suggested that the NPE is the only layer necessary for AH formation as it was observed that the NPE layer alone can maintain normal IOP following selective destruction of the PE layer.

Intercellular junctions

TIGHT JUNCTIONS (ZONULAE OCCLUDENS)
Tight junctions are present between the apical portions of the NPE cells only. These form a barrier for paracellular diffusion and constitute the major component of the blood-aqueous barrier. The existence of a blood-aqueous barrier is evident from the extremely low concentration of protein in aqueous humour. In addition, penetration of horseradish peroxidase into the aqueous humour after intravenous injection is blocked by the tight junctions between the NPE cells. The tight junctions serve two main functions.
Aqueous humour formation

1. they limit the passage of substances through intercellular spaces from the ciliary stroma into the posterior chamber.
2. they maintain the transepithelial potential difference (PD) across the CE.

Green and Pederson found that the CE was extremely permeable to water and suggested that 80 per cent of the aqueous humour was formed by ultrafiltration. Kishida and colleagues showed that the magnitude of the transepithelial PD and tissue resistance (Rt) were low in isolated rabbit iris-ciliary body (ICB) preparations. Based on the findings of electrical parameters in other ‘leaky’ epithelia, they suggested that the CE was also leaky. The convoluted surface profile of CE makes quantification of the surface area difficult. It has been suggested that although the measured electrical parameters are low, the calculated electrical resistance for the unfolded tissue may be high. Moreover, Burstein and co-workers showed that ouabain elicits opposite electrical responses when added to either side of the CE, suggesting that large molecules were not readily diffusible across the CE. In addition, Chu and Candia found that mannitol permeability across the isolated rabbit ICB was comparable to that measured in other ‘tight’ epithelia.

GAP JUNCTIONS

Gap junction channels can mediate direct intercellular communication. Each channel comprises two hemichannels (connexons), each of which is composed of six radially-arranged connexins around a central pole. Using freeze-fracture techniques, intercellular gap junctions have been demonstrated both within each epithelial layer and between the two cell layers. The presence of a large number of gap junctions between PE and NPE cells indicates direct communication between them. The dye coupling between the two cell layers has also been demonstrated using intracellular injection of the fluorescent dye, Lucifer yellow. In addition, the fact that there is no difference in membrane potential and intracellular ion contents between PE and NPE cells indicates that the gap junctions allow free exchange of metabolites and ions and provide electrical coupling between these cells. In other words, the gap junctions can be viewed as a pathway for intercellular communication that facilitates the two cell layers functioning as a syncytium.

ION TRANSPORT ACROSS THE CE

As indicated previously, active transport of solutes across the CE has been generally accepted as the major driving force of AH formation. Although considerable evidence supports the fact that both the PE and NPE cells are involved in AH formation, the functions of these respective cell layers and their interactions in the regulation of formation of AH are yet to be determined. Although the tight junctions in the NPE cell layer may highlight the dominant role of this layer for AH secretion, the presence of a large number of gap junctions between the PE and NPE suggests a coupling of these two layers in transepithelial transport. One of the characteristics of the CE is the asymmetric distribution of ion transporters on the membranes of PE and NPE cells, which is essential for mediating the vectorial ion and solute transport. Several models have been put forward recently to account for ion and solute movement across the bilayer epithelium. Although a unified model is yet to be proposed, the consensus in the literature is that there are at least three transport steps involved in transferring ion and/or solute across the CE. They are illustrated in Figure 2 and are as follow:

1. loading of ion and/or solute from ciliary stroma (blood) into the PE cells across its basolateral border
2. translocation of ion and/or solute through the gap junctions into the NPE cells
3. exclusion of ion and/or solute from NPE cells to the posterior chamber driven by the electrochemical gradient and/or by active transport. TJ: tight junctions, GJ: gap junctions.

Figure 2. Three transport steps are involved in ion and solute transport across the CE:

1. loading of ion and/or solute from ciliary stroma (blood) into the PE cells across its basolateral border
2. translocation of ion and/or solute through the gap junctions into the NPE cells
3. exclusion of ion and/or solute from NPE cells to the posterior chamber driven by the electrochemical gradient and/or by active transport. TJ: tight junctions, GJ: gap junctions.
Na⁺ transport

Na⁺;K⁺-ATPase is an enzyme system, which is responsible for sodium and potassium transport. It is present in all vertebrate epithelia as well as in CE. The Na⁺;K⁺-ATPase consists of two α-subunits and two β-subunits. The α-subunit is the catalytic unit responsible for transport and ATPase activities, whereas the β-subunit facilitates the proper folding of the α-subunit. Under physiological conditions, Na⁺;K⁺-ATPase acts by extruding out three molecules of Na⁺ in exchange for two K⁺ molecules into the cell, at the expense of one ATP. This generates a substantial Na⁺ and K⁺ electrochemical gradient across the plasma membrane that is of vital importance for different cellular functions such as volume regulation, acid-base balance, generation of action potentials and secondary active transport. The activity of the Na⁺;K⁺-ATPase can be blocked by cardiac glycosides such as ouabain.

Na⁺;K⁺-ATPase is a prerequisite for AH formation and has been histochemically identified in the CE of rabbit[60,64 and ox[65. Moreover, it has been functionally expressed in the CE of different species such as rabbit[18,32 cat[66 ox[25,53 monkey[67 and human. The electrogeneric nature of this enzyme has been demonstrated by the depolarising effect of ouabain on the transepithelial PD[22,68,69 and membrane potential[46,69 in different species. Na⁺;K⁺-ATPase was mainly found along the basolateral infoldings and interdigitations of both the PE and NPE cells[65,70 and higher activities were found on the NPE cells. Dunn, Lyle and Crook[29 recently demonstrated that the quantity of catalytic α-subunit of Na⁺;K⁺-ATPase in bovine NPE cells was approximately eight times more than that in PE cells. In addition, the Na⁺;K⁺-ATPase of PE cells probably differs functionally from that of NPE cells as reflected by the different isoforms shown in these two cell types. It has been proposed that the Na⁺;K⁺-ATPase of PE cells is not involved in the transepithelial Na⁺ transport but plays a role in maintaining the intracellular ionic balance.[71,72 Under physiological conditions, the Na⁺;K⁺-ATPase of PE cells possibly acts as a ‘housekeeping’ ATPase to maintain the transmembrane Na⁺ and K⁺ gradients, which may serve as energy sources to transport other ions against their electrochemical gradients. Na⁺;K⁺-ATPases exist in different isoforms and in the CE, the distribution of different isoforms of Na⁺;K⁺-ATPase has been shown. For example, in NPE cell layer, both α- and β-isoforms in the region of the pars plicata were found to be higher than in the region of the pars plana. This finding is in good agreement with the notion that the pars plicata plays a dominant role in AH secretion. The measurement of transepithelial PD across the ciliary body provides a direct way to study the ion transport characteristics of the CE. Pioneering work in this field was done by Cole[72,75 who used an in vitro preparation in the Ussing chamber. He detected a PD, with aqueous side positive, across the ciliary body of ox and rabbit. Replacing the bathing Na⁺ with either choline or mannitol decreased the magnitude of the PD. Cole[76 also observed a positive PD in vivo by inserting an electrode into the posterior chamber of the rabbit eye. He then put forward the ‘standing gradient osmotic flow’ model, also proposed by Diamond[77 and postulated that active Na⁺ transport was involved in aqueous humour secretion. In his hypothesis, the active exclusion of Na⁺ by the Na⁺;K⁺-ATPase at the basolateral surface of NPE cells caused a local accumulation of Na⁺ at the intercellular clefts and in the invaginations of the basal cell membrane. This would generate a local hyperosmotic environment, which would subsequently drive water into the clefts and invaginations, resulting in the formation of AH. Simultaneously, the positive PD also tended to attract anions into the AH.[29 The hypothesis of active Na⁺ transport was further supported by several studies on the rate of Na⁺ accession from blood to the posterior chamber. In dog, it has been shown that the Na⁺ accession rate was approximately equal to the fluid formation rate.[78 Intraocular administration of ouabain reduced Na⁺ accession and AH formation by 30 to 50 per cent in species such as cat,[79 dog[80 and monkey[81. However, Maren[82 did not detect the reduction of Na⁺ accession with ouabain in dogfish. He suggested that the Na⁺ secretion might be masked by the large diffusional Na⁺ flux in this species, rendering the reduction insignificant. Furthermore, ouabain has been shown to reduce IOP in experimental animals,[77,83 probably primarily by inhibiting AH secretion and not impeding the outflow facility.[79,80]

Despite this evidence, the notion of primary Na⁺ transport across the CE has been questioned by subsequent electrophysiological studies that observed a negative PD across the CE.[22,23,67,69,84 The negative PD suggested an active anion (possibly HCO₃⁻ and/or Cl⁻) rather than Na⁺ dominates transport system. This discrepancy can be explained by two possibilities:

1. The bathing solution used in Cole’s studies was nominally HCO₃⁻ free. It was found that depletion of HCO₃⁻ from the bathing medium depolarised and reversed the polarity of the PD.[22,40
2. It was shown that the PD generated by the retinal pigment epithelium was positive on the retinal side,[83,85 which could have influenced the accuracy of the PD measured by inserting an electrode into the posterior chamber.[69

The absence of Na⁺ transport was further confirmed by several electrophysiological studies, in which no significant net Na⁺ flux was detected across the ciliary body of rabbit,[73,86 cat[84 toad[87 and more recently, ox.[90 Holland[61 found a net Na⁺
transport in the cat eye but this seems to be driven by the residual transepithelial PD. When the residual PD was corrected, no statistically significant net Na⁺ flux was demonstrated. Moreover, it was found that ouabain did not inhibit but slightly stimulated the unidirectional blood-to-aqueous and aqueous-to-blood Na⁺ flux, when applied to one or both sides of the preparation, even though a marked inhibitory effect on the electrical parameters was observed. These findings suggest that the unidirectional Na⁺ fluxes are high; therefore, a small difference between these fluxes may be difficult to detect. Candia, Shi and Chu suggested that the net Na⁺ transport might have been masked by the large diffusional and/or bi-directional Na⁺ fluxes. To minimise the effect of diffusional fluxes, they measured the unidirectional Na⁺ fluxes by reducing the bathing Na⁺ concentration. At 30 mM, a statistically significant net Na⁺ transport in the blood-to-aqueous direction was found. However, the measured Na⁺ flux was not sufficient to account for the rate of AH formation in vivo.

**HCO₃⁻ transport**

It has long been believed that active HCO₃⁻ transport is involved in AH formation and carbonic anhydrase (CA) plays a crucial role, either directly or indirectly, in regulating the HCO₃⁻ movement across the CE. CA is an important enzyme that presents in most secretory epithelia. It catalyses the reversible hydration of carbon dioxide:

\[ \text{CO}_2 + \text{H}_2\text{O} \xleftrightarrow{\text{CA}} \text{H}_2\text{CO}_3 \xrightarrow{\text{H}^+ + \text{HCO}_3^-} \]

There are at least seven isozymes of mammalian CA (CA I to CA VII) cloned and sequenced with different kinetic properties, susceptibility to inhibitors, intracellular location and tissue distribution. CA is involved in a variety of physiological functions such as pH regulation, CO₂ and HCO₃⁻ transport and water and electrolyte balance.

Friedenwald was the first to propose the role of CA in AH formation. He postulated that the splitting of water produced H⁺ and OH⁻. The CA facilitates the buffering of OH⁻ by CO₂, resulting in a formation of HCO₃⁻ which subsequently secretes into the AH. The HCO₃⁻ secretion was electrically neutralised by the entrance of Na⁺ from the blood plasma. Consistent with this finding, Kinsey and Reddy demonstrated that the accession rate of HCO₃⁻ to the posterior chamber was extremely rapid and the administration of acetazolamide decreased the concentration and accumulation of HCO₃⁻ in the AH.

Friedenwald’s hypothesis was further supported by the studies of the steady-state HCO₃⁻ concentration in the aqueous humour compared to plasma concentrations. It has been shown, in species like rabbit and guinea pig, that the HCO₃⁻ concentration in aqueous humour was higher than that of plasma. The excess of HCO₃⁻ was usually accompanied by a deficit of Cl⁻ in the AH. These observations tend to support the hypothesis of active HCO₃⁻ transport into the eye, which results in an alkaline AH. However, this was not necessarily true for all species studied. In primates such as monkey and human, the AH was acidic with a deficit of HCO₃⁻ and an excess of Cl⁻. Becker showed that systemic administration of acetazolamide produced opposite effects in these two species. In rabbit, it reduced the aqueous-to-plasma ratio of HCO₃⁻ but increased the ratio of Cl⁻, whereas in human, it decreased the aqueousto-plasma ratio of Cl⁻ but increased the ratio of HCO₃⁻. They suggested that different transport mechanisms might be operating in different species. The fact is that the administration of carbonic anhydrase inhibitor (CAI) reduces AH formation and lowers the IOP in a number of experimental animals and in human, without significant effect on outflow facility. Because of this effect, CAIs have been used in glaucoma treatment to reduce the IOP for several decades. Scientists have been trying hard during this time to understand the detailed physiological relationship between CAIs and AH formation.

Becker put forward Friedenwald’s hypothesis, which suggested a role for CA in AH production of these different animal species. In rabbit, the hydration of CO₂ catalysed by CA, generates HCO₃⁻ which is subsequently secreted into the AH. The residual H⁺ may be reabsorbed into the ciliary stroma by Na⁺ exchange. This explains the alkaline pH and the high concentration of HCO₃⁻ in rabbit AH. In human, the process occurs in the reverse direction. The HCO₃⁻ generated from CO₂ hydration is exchanged with Cl⁻ in blood plasma, while the residual H⁺ may enter the eye or be exchanged with Na⁺ in the blood plasma. This accounts for the acidic pH and high Cl⁻ in human AH. Later, Cotlier proposed the presence of a HCO₃⁻-ATPase in the CE, which provided the energy for the HCO₃⁻ transport in species with alkaline pH or Cl⁻ transport in species with acidic pH. The administration of acetazolamide lowered the activity of HCO₃⁻-ATPase and thereby reduced HCO₃⁻ secretion in rabbit or Cl⁻ secretion in human.

Maren later questioned the use of steady-state concentrations of ions when studying AH formation. He suggested that the HCO₃⁻ concentration in the AH varied dramatically among vertebrate eyes, which might not be due to the operation of different transport mechanisms. As the measured ionic concentrations in AH are the result of interactions with metabolites of the surrounding intraocular tissues, it might not necessarily provide any insight into the processes of secretion. He suggested that it was more important to measure the HCO₃⁻ accession rate rather than the steady-state HCO₃⁻ concentration in AH. Later, by measuring the ionic accession from blood plasma to the posterior chamber in dog, a species with a slight excess of both HCO₃⁻ and Cl⁻ in the aqueous, Maren found that acetazolamide reduced the fluid secretion with a concomitant lowering of the accession rate for both Na⁺ and HCO₃⁻ but not of Cl⁻. Based on similar reductions in their accession rates, the movement of Na⁺ and HCO₃⁻ might be coupled. A year later, Maren found comparable results in monkey except that Cl⁻ accession was also reduced by 25 per cent.

Electrophysiological studies of rabbit ICB have demonstrated a negative PD in the presence of HCO₃⁻ in the bathing solution. Depletion of HCO₃⁻ from the bathing media resulted in a reversal of PD.
which suggested that HCO$_3^-$ was essential to the maintenance of electrical parameters. In experiments with intracellular pH (pHi) regulation and Na$^+$ uptake measurements, a Cl$^-$-dependent, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive Na$^+$-H$^+$ cotransporter was found in cultured bovine PE cells. Later, Butler also demonstrated a Na-dependent HCO$_3^-$ uptake in rabbit PE cells. This antiporter provides a route for HCO$_3^-$ accumulation in CE. Together with Na$^+$-H$^+$ antiporter, they in turn facilitate the regulation of intracellular pH and transepithelial transport. Functional evidence for the presence of Cl$^-\text{-HCO}_3^-$ antiporter at the basolateral membrane of NPE cells has also been demonstrated and may provide an efflux pathway for HCO$_3^-$ to the posterior chamber. Although transepithelial secretion of HCO$_3^-$ was found in both PE and NPE cells of human clinical specimens, a Cl$^-$-dependent, 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS)-sensitive Na$^+$-H$^+$ cotransporter was found in cultured bovine PE cells. Later, Butler also demonstrated a Na-dependent HCO$_3^-$ uptake in rabbit PE cells. This antiporter provides a route for HCO$_3^-$ accumulation in CE. Together with Na$^+$-H$^+$ antiporter, they in turn facilitate the regulation of intracellular pH and transepithelial transport. Functional evidence for the presence of Cl$^-\text{-HCO}_3^-$ antiporter at the basolateral membrane of NPE cells has also been demonstrated and may provide an efflux pathway for HCO$_3^-$ to the posterior chamber.

The presence of CA was demonstrated by histochemical methods in the CE of rabbit, monkey and human. Consistent with these findings, a number of biochemical studies also detected the activities of CA in the CE of different species. In CE, both cytosolic CA II and membrane-bound CA IV have been identified and the co-ordinated effect of these two isozymes may give rise to a net transepithelial HCO$_3^-$ transport. CA II was found in both PE and NPE cells of human CE by immunological techniques. Ridderstrale studied the localisation of the CA IV in CA II-deficient mouse. CA IV was abundantly detected at the apical and basolateral membranes of both PE and NPE cells and it was shown that benzolamide, which does not readily penetrate the plasma membrane, caused a larger inhibition of PD when added to the aqueous side than to the blood side. They suggested that CA IV at the basolateral membrane of NPE cells played a dominant role in catalytic action. In subsequent studies, the functional role of the CA IV at the basolateral membrane of NPE cells was addressed. It was shown that the CA IV facilitated the conversion of HCO$_3^-$ and H$^+$ into CO$_2$ and H$_2$O. On the inhibition of CA IV by membrane impermeable dextran-bound CAI (DBI), the pH$_r$ was reduced, which might be due to the inhibition of Na$^+$-H$^+$ antiport at the basolateral membrane of NPE cells. Although considerable evidence regarding the functional role of different CA isozymes has been presented, the transepithelial HCO$_3^-$ transport across the CE has not been directly shown. The difficulty lies in the dynamic conversion of bicarbonate ions to carbon dioxide, which has rendered it difficult to study. Candia designed a closed circulating chamber, which allows the accurate control of such conversion. We have studied bicarbonate flux using this chamber and still failed to demonstrate any significant bicarbonate transport by the CE. Therefore, the bicarbonate ion and carbonic anhydrases are likely to exert their effects on AH formation through local modulation of chloride secretion (see below) by pH regulation or transmembrane transport.

**Cl$^-$ transport**

Compared to HCO$_3^-$ transport, the notion of an active Cl$^-$ transport has only recently received attention. Cole suggested that, in addition to Na$^+$ transport, active Cl$^-$ transport might also occur across the CE. Ouabain, which blocks the Na$^+$-K$^+$-ATPase, produced a reduction of Na$^+$ and Cl$^-$ transport from the blood plasma to the posterior chamber and a concomitant reduction of AH formation in cat. Kishida and colleagues showed that the reduction of Cl$^-$ accession by 25 per cent with acetazolamide in monkey, a species with an excess of Cl$^-$ and deficit of HCO$_3^-$ in the AH. However, in other species such as dogfish and dog, no reduction of Cl$^-$ accession with acetazolamide was demonstrated. Wiedenholt and Zadunaisky showed that the intracellular Cl$^-$ activity in shark CE was significantly higher than that predicted from the electrochemical equilibrium, indicating that there was a mechanism for active Cl$^-$ accumulation into the cells. The mechanism of Cl$^-$ uptake was mediated through a furosemide-sensitive pathway as the level of intracellular Cl$^-$ activity was significantly decreased when furosemide was applied to the bathing solution. The reduction of intracellular Cl$^-$ activity with furosemide was also demonstrated in frog retinal pigment epithelium.

In electrophysiological studies, a clear dependence of bathing Cl$^-$ concentration on the short-circuit current (I$_{sc}$) was demonstrated in the ciliary body of cat, toad and rabbit. In addition, it has been shown that furosemide reduces the I$_{sc}$ in the ciliary body preparation of dog, toad and rabbit. However, neither a Cl$^-$-dependent nor furosemide-sensitive I$_{sc}$ was demonstrated in other electrophysiological studies in rabbit. Measurement of transepithelial Cl$^-$ fluxes across the CE also produces conflicting results. A substantial net Cl$^-$ transport in the blood-to-aqueous direction was demonstrated in cat, toad, rabbit and ox.

Kishida and colleagues showed that the net Cl$^-$ flux was larger than the measured I$_{sc}$, suggesting the presence of net transport of other ions across the CE. Pesin also found that the electrical conductance calculated from unidirectional fluxes was about 2.7 times larger than that from measured conductance, although they did not find any net Na$^+$ and Cl$^-$ transport. These results suggest the presence of electrically silent transport mechanisms. Helbig and co-workers showed that the uptake of Na$^+$ and Cl$^-$ in cultured bovine PE cells was coupled and stimulated by the presence of the other ion. For example, the Na$^+$ uptake into the NaCl-depleted PE cells was stimulated by the presence of Cl$^-$ in the bathing media and vice versa.

The investigation of ionic transport across the CE is difficult because the ciliary body has a complex anatomy, with its fragile processes and double-layered epithelium. To study the properties of PE and NPE cell layers independently, tissue culture techniques have been developed to grow the respective cell layers separately. Based on the results of these studies, Wiedenholt, Helbig and Korbmacher proposed that there were two major mechanisms of coupled NaCl uptake into the PE cells. Figure 3 illustrates the possible pathways for their uptake into the functional syncytium. One of the mechanisms was via bumetanide-sensitive Na$^+$-K$^+$-2Cl$^-$ cotransporter and the other mechanism was via parallel Cl$^-$-HCO$_3^-$ and Na$^+$-H$^+$ double exchangers/antiports. Both of these pathways allowed for the
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uptake of Na⁺ and Cl⁻ into the CE in an electroneutral manner.

**NA⁺-K⁺-2CL⁻ COTRANSPORTER OR SYMPORT**

The Na⁺-K⁺-2Cl⁻-cotransporter has been well characterised in a variety of epithelia concerned with secretion and absorption. It transports Na⁺, K⁺ and Cl⁻ into and out of cells in an electrically neutral manner, in most cases with a stoichiometry of 1 Na⁺, 1 K⁺ and 2 Cl⁻. It plays a vital role in a number of physiological functions such as secretion, absorption and cell volume regulation.

Furosemide and bumetanide are loop diuretics that can inhibit the activity of Na⁺-K⁺-2Cl⁻-cotransporter. Furosemide has been shown to have a profound effect on electrical parameters and intracellular Cl⁻ activity of the CE, suggesting the involvement of the Na⁺-K⁺-2Cl⁻-cotransporter in transepithelial transport. Helbig and associates showed that the uptake of ²²Na⁺ and ³⁶Cl⁻ in cultured bovine PE cells was inhibited by bumetanide. In addition, it has been demonstrated that bumetanide caused a volume reduction in PE but not NPE cells, indicating a net loss of cellular electrolytes and water. Reducing the bathing Na⁺, K⁺ and Cl⁻ concentrations iso-osmotically caused shrinkage of PE cells that could be inhibited by bumetanide. This evidence supports the hypothesis that PE cells possess a bumetanide-sensitive Na⁺-K⁺-2Cl⁻ symport. In addition to PE cells, this cotransporter has also been detected in NPE cells. Very recently, Dunn showed that the immunofluorescence staining of Na⁺-K⁺-2Cl⁻-cotransporter was intense along the basolateral membrane of bovine PE cells, whereas NPE cell showed only a diffuse and cytoplasmic staining. In addition, Do and To have demonstrated that bumetanide inhibited over 80 per cent of the chloride secretion across the bovine CE in vitro. These data suggest that Na⁺-K⁺-2Cl⁻-cotransporter is likely to be the dominant NaCl uptake mechanism in ox.

**CL⁻-HCO₃⁻ AND NA⁺-H⁺ DOUBLE EXCHANGERS OR ANTIPORTS**

Parallel Cl⁻-HCO₃⁻ and Na⁺-H⁺ double exchangers are present in virtually all cells and are important to a number of vital physiological functions such as the pH, regulation, cell volume regulation and transepithelial transport. Functional evidence for the presence of these exchangers has been demonstrated in PE cells and NPE cells. It has been shown that these exchangers are involved in the regulation of pH in the CE. In cultured bovine PE cells, the Na⁺-H⁺ exchanger was shown to regulate the pH during steady-state conditions and after acid load, while Cl⁻-HCO₃⁻ exchanger was responsible for regulating the pH after alkali load. In addition, a DIDS-sensitive Na⁺-HCO₃⁻ cotransporter was also shown to regulate the pH during steady-state conditions and after acid load in the same cells. Wiederholt, Helbig and Korbacher suggested that the NaCl uptake into the functional syncytium could be mediated through the parallel Na⁺-H⁺ and Cl⁻-HCO₃⁻ exchangers, which were functionally coupled to CA and pH. They proposed that CA IV facilitated the dehydration of carbonic acid in the ciliary stroma and thereby the CO₂ could diffuse into the PE cells through the basolateral membrane.

Cellular metabolism provided another source for intracellular CO₂. The hydration of endogenous CO₂ by CA II provided the HCO₃⁻ and H⁺ as substrates for the Cl⁻-HCO₃⁻ and Na⁺-H⁺ exchangers at the basolateral membrane of PE cells. In this model, a net NaCl uptake was achieved from the ciliary stroma into the PE cells, while HCO₃⁻ and H⁺ were recycled across the basolateral membrane of PE cells. In this case, there was an absence of transepithelial HCO₃⁻ transport, although it had a central role in mediating NaCl transport. The model was later supported.

**Figure 3. Diagram showing the possible pathways for the uptake of Na⁺ and Cl⁻ across the CE: (A) via Na⁺-K⁺-2Cl⁻ cotransporter and (B) via Cl⁻-HCO₃⁻ and Na⁺-H⁺ double exchangers. Both pathways allow the uptake of Na⁺ and Cl⁻ into the functional syncytium in an electroneutral manner. The energy for the accumulation of Cl⁻ in CE is provided by the transmembrane Na⁺ gradient maintained by the activity of Na⁺,K⁺-ATPase. TJ, tight junctions; GJ, gap junctions.**
by studies of the intracellular ion contents of CE cells using the technique of electron probe X-ray microanalysis. Both PE and NPE cells contained more Cl when incubated with HCO\textsubscript{3}\textsuperscript{-}-rich solution. In the presence of acetazolamide, the increase of Cl\textsuperscript{-} content was inhibited. This suggested that the reduction of Cl\textsuperscript{-} content was due to the inhibition of NaCl uptake through the parallel Na\textsuperscript{+}H\textsuperscript{+} and Cl\textsuperscript{-}HCO\textsubscript{3}\textsuperscript{-} double exchangers.

**Cl Channels**

NaCl taken up by the PE cells has to transfer to NPE cells so that it can exit into the posterior chamber. In NPE cells, Na\textsuperscript{+} may be excreted into the posterior chamber by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, which pumps out Na\textsuperscript{+} in exchange for K\textsuperscript{+} in the AH. The K\textsuperscript{+} recycles through K\textsuperscript{+} channels and Cl\textsuperscript{-} passively diffuses through Cl channels into the AH.

In theory, the rate-limiting step of transepithelial transport could be at one of three locations as shown in Figure 2:

1. the basolateral membrane of PE cells
2. gap junctions
3. the basolateral border of NPE cells

Civan and co-workers\textsuperscript{50} suggested that the Cl\textsuperscript{-} channel at the basolateral surface of NPE cells was the most likely limit on the rate of AH formation. The reasons were:

1. The intracellular Cl\textsuperscript{-} activity was shown to be significantly higher than that predicted from electrochemical equilibrium, suggesting that the uptake of Cl\textsuperscript{-} from the ciliary stroma might not be rate-limiting.
2. The membrane potential\textsuperscript{46} and intracellular ion contents\textsuperscript{48} between the PE and NPE cells were similar, indicating an unimpeded ion flow between the PE and NPE cells through gap junctions.
3. Under baseline conditions, the activities of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and permeability of K\textsuperscript{+} channel at the basolateral membrane of NPE cells were high, suggesting that they were not rate-limiting.\textsuperscript{50,51}

We have also observed a strong inhibition of chloride flux in ox when 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) was applied to NPE cells.\textsuperscript{137}

It has been suggested that the mechanism of regulatory volume decrease (RVD) also may be responsible for AH formation.\textsuperscript{144} RVD is the cellular response to osmotic swelling of the cell and it reveals the ion transport machinery and water pumping activities of a cell. Yantorno and colleagues\textsuperscript{46} studied a whole cell patch clamp preparation on anisosmotic cell swelling using cells (ODM C1-2/SV40) derived from human NPE. They found that K\textsuperscript{+} conductance but not Cl\textsuperscript{-} conductance, provided a major contribution to the whole cell conductance under baseline isotonic conditions. However, cell swelling induced by hypotonicity triggered RVD, which was dependent primarily on an increase in Cl\textsuperscript{-} channel activity.\textsuperscript{145,146} These data on RVD can provide useful information on the efflux pathway of chloride ions, which constitutes the final step in transepithelial chloride movement. Moreover, protein kinase C (PKC) downregulated Cl channels in cultured human NPE cells, while staurosporine, an inhibitor of PKC, was shown to stimulate the RVD\textsuperscript{147} and upregulate the whole cell Cl\textsuperscript{-} currents isometrically.\textsuperscript{57} The kinetic properties of the staurosporine-activated Cl\textsuperscript{-} channels were similar to those of the volume-activated Cl\textsuperscript{-} channels, suggesting that cystic fibrosis transmembrane conductance regulator (CFTR) or Cl\textsuperscript{-} channel regulator pICln could be the possible candidates for channel activity. However, the mRNA for CFTR cannot be detected by Northern analysis in both the cultured NPE cells and ciliary body tissue while a high level of pICln transcripts was observed by polymerase chain reaction. Consistent with this finding, McCannel and associates\textsuperscript{148} showed that patients with cystic fibrosis, in which the function of CFTR was defective, displayed a normal circadian pattern of AH formation and were responsive to timolol treatment. These results suggest that CFTR does not play an important role in regulating the Cl secretion in AH formation. Coca-Prados and colleagues\textsuperscript{49} later demonstrated the expression of chloride channel CIC-3 transcripts in human NPE cells by polymerase chain reaction. Civan\textsuperscript{150} suggested that CIC-3 might be regulated by the pICln that provided the same conduit for both volume-activated and isotonically staurosporine-activated Cl channels of human NPE cells. Chen, Wang and Jacobs\textsuperscript{52} also found the presence of pICln protein in native bovine NPE cells. They suggested that the intrinsic pICln played an important role in the activation pathway of volume-activated Cl\textsuperscript{-} current and cell volume regulation using the antisense oligonucleotide technique. However, until recently, Sanchez-Torres and colleagues\textsuperscript{145} showed that neither a translocation of pICln from the cytoplasm into the plasma membrane nor changes in pICln expression at the protein level were observed when the NPE cells were subjected to hypotonic treatment. Instead, a moderate decrease (approximately 30 per cent) in pICln mRNA expression on cell swelling of NPE cells was found. They suggested that the pICln might not be on the plasma membrane of NPE cells and its effect on CIC-3, if any, was remote. In addition, Wang, Chen and Jacobs\textsuperscript{53} detected the presence of immunofluorescence for CIC-3 protein in both the plasma membrane and cytoplasm of bovine NPE cells but with the predominant localisation within the nucleus. They found that the activation of volume-activated Cl current was delayed and the current was diminished by the antisense inhibition of endogenous CIC-3 treatment. However, the CIC-3 was not the sole Cl\textsuperscript{-} channel, as it could account for only about 60 per cent of the total volume-activated current in NPE cells, indicating that there must be other Cl\textsuperscript{-} channels that contributed to this current.

**Ascorbic acid transport**

It is well known that the concentration of AA in the AH is significantly higher than that of the blood plasma in many species including human. In rabbit eye, the concentration of AA in the anterior and posterior chambers is approximately 50- to 70-fold higher than that in the blood plasma.\textsuperscript{10} Reiss and associates\textsuperscript{8} studied the AA concentration in AH in 22 mammalian species and found that the diurnal animals had a higher level of AA than nocturnal animals. They suggested that the AA in the aqueous humour might be used to protect the eyes against photo-oxidative damage.

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The secretion of AA in AH requires an active transport of AA across the CE. Helbig and co-workers\(^2\) studied the uptake of AA in cultured bovine PE cells and showed that the intracellular concentration of AA was about 40 times higher than in the extracellular media. The accumulation of AA was Na\(^+\)-dependent and the kinetic properties suggested a stoichiometry of two or more Na\(^+\) for one AA. In addition, it has been shown that the uptake of AA can be reduced by metabolic inhibitors, hypothermia and reduced bathing Na\(^+\) concentration, indicating that the uptake process is linked to metabolism and coupled to the Na\(^+\) transport across the plasma membrane.\(^2,155\) The coupling of AA to the inward Na\(^+\) gradient not only provides the energy required for the secretion of AA against their concentration gradient but also enhances the transepithelial Na\(^+\) transport.\(^4\)

Active transepithelial AA transport occurs across the isolated rabbit\(^2\) and bovine\(^13\) CE. The transport of AA is via a Na\(^+\)-dependent carrier-mediated process. In addition, Chu and Candia\(^2\) suggested that the measured rate of AA transport is not sufficient to maintain the normal AA concentration in the AH observed in vivo. Later, Mead and colleagues\(^156\) examined the transepithelial AA transport across the isolated rabbit ciliary epithelial bilayer. When a higher bathing AA concentration was used, they were able to demonstrate a net transport of AA that could account for the AA concentration in vivo.

**REGULATION OF AQUEOUS HUMOUR FORMATION**

IOP is not constant but displays diurnal variation. The diurnal fluctuation of IOP is observed both in normal and glaucomatous subjects although the latter group displays a more pronounced variation. The variations of IOP in a day undoubtedly reflect a change of AH dynamics. It has been shown that the rate of AH formation is 2.61 \(\mu\)l/min\(^2\) during the day and 1.08 \(\mu\)l/min\(^1\) at night in normal human subjects.\(^157\) This circadian rhythm strongly indicates that the AH secretion is precisely regulated although the mechanism of the regulation is not fully understood.

The \(\beta\)-adrenergic system has long been recognised to play a vital role in regulating AH formation. The presence of \(\beta\)-adrenergic receptors has been demonstrated in the ciliary process of rabbit,\(^158\) \(\alpha\)\(^x\)\(^159\) and human.\(^160\) Other studies have revealed that the \(\beta\)-adrenergic receptors are predominantly in \(\beta_2\)-subtype.\(^160-163\) The administration of timolol, a \(\beta\)-adrenergic antagonist, either systemically or topically reduces AH formation and lowers IOP in normal human subjects.\(^155,164,166\) Clinical treatment of glaucoma has been most successfully directed towards reducing AH formation with \(\beta\)-blockers. In addition, Shahidullah, Wilson and Millar\(^166\) showed that timolol caused a concomitant reduction of AH formation and IOP in arterioles-perfused bovine eye.

Several signal transduction pathways have been suggested to be responsible for the regulation of aqueous flow. The secondary messengers such as cyclic nucleotides and Ca\(^2+\) may play important roles in these cascades by eliciting different cellular responses. It is well known that the vasoactive intestinal peptide (VIP) as well as the \(\beta\)-adrenergic system are directly coupled to adenylylcyclase (AC) in ciliary processes.\(^25\) When the catecholamines (agonists) bind to the receptors on the plasma membrane, they stimulate the AC via guanyl nucleotide binding protein (G-protein), resulting in the conversion of ATP to cyclic adenosine monophosphate (cAMP). The cAMP then activates the cAMP-dependent enzyme known as protein kinase A which subsequently induces different cellular responses via protein phosphorylation.

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**Figure 4. Signaling transduction and ions transport of the CE.** VIP (vasoactive intestinal peptide) binds to receptor and leads to an activation of adenylate cyclase (AC) via G-protein (G). AC increases the conversion of ATP to cyclic adenosine monophosphate (cAMP). cAMP then activates the protein kinase A which subsequently induces different cellular responses via protein phosphorylation.
onic conditions. These findings imply that stimulation of cAMP formation may be associated with an increase of transepithelial ion transport, which increases the AH formation. The β-adrenergic antagonists block the stimulation of cAMP production and therefore lead to a reduction of aqueous humour formation and IOP.

However, contrasting evidence has been presented. It has been shown that topical and intravital forskolin reduces the IOP in rabbit, monkey and human. Lee and co-workers found that topical forskolin caused a reduction of IOP via an inhibition of AH formation in monkey. Furthermore, other studies showed that topical forskolin or isoproterenol increased the concentration of cAMP in the aqueous humour along with a reduction of AH formation and IOP in rabbit. In human, topical application of isoprot-

In vivo pharmacological spectrum of adrenergic agonists can increase parallel Cl--HCO3 exchange transport in individual epithelial cell layer. Third, the regulation of chloride transport in the NPE and its role in secreting AH. In addition, we can now propose some candidate protein transporters and channels for chloride movement across the membranes of the CE. In brief, the influx pathways of sodium and chloride ions involve Na‘K‘-2Cl- cotransporter, Na‘H‘ and Cl-HCO3 double exchangers. The ions are then diffused through the gap junctions between the PE and NPE. Chloride ion leaves the NPE via chloride channel and sodium via the Na‘K‘-ATPases (Figure 3).

However, the story of AH formation is still far from clear. First, the in vitro AH secretion rate estimated from ion flux studies using isolated CE bilayer is far lower than the measured in vivo rate. The factors underlying the in vitro and in vivo differences are yet to be studied and determined. Second, the model describing the movement of NaCl across the CE is incomplete. NaCl uptake at the PE is not fully characterised. The contribution of parallel Cl-HCO3 and Na‘H‘ double exchangers versus Na‘K‘-2Cl- cotransporter is one of the most heated debates in AH research. It is likely that both groups of transporters play a role in sodium chloride movement and their relative contributions also may vary in different species. We still have not identified the exit pathway or the chloride channel at the NPE cells. Third, the regulation of chloride transport in individual epithelial cell layer as well as in the bilayer is complex and requires further study.

For many years, we have been blessed with a number of fairly effective anti-glaucoma drugs without knowing how these drugs work, and with the containment of the glaucoma problem without understanding how AH is formed. The rationale behind our understanding of the ion transport mechanism in AH formation and its regulation is to provide a scientific framework to design better drugs that can target specifically the key protein players in AH formation. For example, we may be able to improve the potency of the drugs that bind to particular transporters or channels more tightly and hence minimise the need to administer anti-glaucoma eye drops frequently. Together with drugs that target the anterior drainage pathway, we might be able to provide better clinical tools for eye care professionals to tackle the glaucoma problem and help many millions of glaucoma suffers worldwide.

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