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Epithelial Potassium Transport: Tracer and Electrophysiological Studies in Choroid Plexus

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Summary. Transport of potassium by bullfrog choroid plexus was studied using tracers and ion-selective microelectrodes.

1) Tracers: We measured unidirectional uptakes of ^{42}K across each surface of the plexus, efflux of ^{42}K from loaded tissues, intracellular potassium pools, and [^{3}H]-ouabain binding. ^{42}K uptake across the brush border membrane was composed of diffusional and saturable components. The saturable component exhibited kinetics of a two-site model with K_m's of 0.3 mM and a V_{max} of 8 μmol cm^{-2} hr^{-1}. Ouabain inhibited uptake across the brush border membrane with a K_i of 1×10^{-7} M. Ethacrynic acid, phloretin, amiloride, and low sodium concentrations inhibited uptake, whereas bicarbonate ions increased transport up to 100%. The rate of ouabain-sensitive uptake across the serosal surface was only 6% of that across the ventricular surface. The efflux of potassium across the brush border membrane could account for most of the efflux from the epithelium. Potassium was accumulated within the plexus to a concentration in excess of 100 mM. ^{42}K in the extracellular compartments exchanged with 40–55% of the intracellular potassium. Ouabain bound to the brush border membrane with a K_m of 8×10^{-7} M, a k_1 of 3.8×10^{4} mol^{-1} min^{-1} and a k_{-1} of 3×10^{-2} min^{-1}. Ouabain binding was blocked by cymarin and gitoxigin.

2) Electrodes: Under control conditions the intracellular electrical potential, E^{vc}, was −45 mV and the apparent intracellular K^+-concentration, K_c^+, was 90 mM. K^+ ions appeared to be actively accumulated within the epithelium. E^{vc} and K_c^+ were followed under three experimental conditions: (i) treating the tissue with ouabain; (ii) varying the ventricular K^+ concentration; and (iii) passing transmural currents. It is concluded that the permeability of the ventricular membrane to potassium ($1–5 \times 10^{-5}$ cm sec^{-1}) was much greater than the permeability of the serosal membrane and that the rate of K pumping into the epithelium was $0.35–0.55 \times 10^{-9}$ mol cm^{-2} sec^{-1}. 96% of the transmural current was paracellular, and the resistance of the serosal membrane was nine times greater than that of the ventricular membrane. Increasing K_c^+ produced Nernstian changes in E^{vc} and E_K^{vc}, but the cells only depolarized by 0.13 mV for each mV increase in the chemical potential of the ventricular solutions.

Both types of experiments are consistent with a model of the epithelium where the cells are predominantly K^+-permeable (at the ventricular membrane) and there is a large paracellular shunt. K^+ is actively transported into the cells by a brush border Na/K-pump, only to leave the cell passively across the same membrane. The pump, which is electrogenic, transports two K ions into the cell for every three Na ions pumped out. There are about 10×10^{6} pumps/cell, and each pump turns over 10 times/sec. The choroid epithelium behaves as predicted by the model proposed first by Koefoed-Johnsen, V., Ussing, H.H. (1958) *Acta Physiol. Scand.* **42**:298.

The frog choroid plexus secretes cerebrospinal fluid (CSF) into the ventricles of the brain from the blood (*see* Wright, 1978*a*, for a recent review). The morphology of the epithelium resembles that of other simple epithelial tissues (Fig. 1). The brush border membranes faces the CSF in ventricles of the brain, whereas the basolateral membranes face the serosal (or vascular) compartment. The epithelium is composed of a single layer of cuboidal cells joined together at their apical surface by so-called tight junctions. There is substantial evidence that the junctions represent a major pathway for the transport of small ions (Na^+, Cl^- and K^+) across the epithelium. The epithe-

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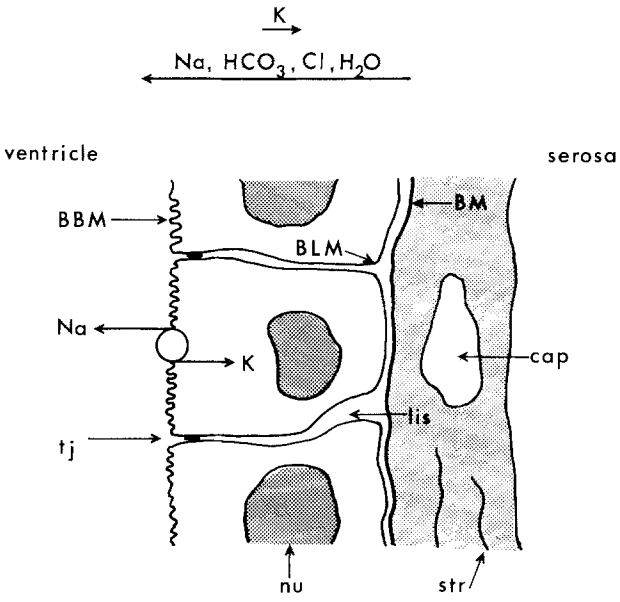

Fig. 1. A drawing of the choroid plexus epithelium. The cuboidal cells are joined at their apical surface by tight junctions (*tj*) and rest on a basement membrane (*BM*) and thin stroma (*str*) of connective tissue rich in capillaries (*cap*). The surface facing the CSF in the ventricles is called the brush border membrane (*BBM*) or ventricular membrane, while that facing the serosal compartment is the basolateral membrane (*BLM*) or serosal membrane. Na, HCO_3, Cl and H_2O are transported from serosa to CSF, and there is a small net reabsorption of K from the CSF into blood

lium rests on a basement membrane and a thin stroma of collagen fibers, fibroblasts, and blood vessels. 25–40% of the cells in the rat choroid plexus are epithelial cells and they occupy 65–95% of the total cell volume. The cytoplasm of the epithelium contains a large, central nucleus and numerous mitochondria concentrated near the brush border membrane. These subcellular organelles occupy 20–50% of the cell volume.

The direction of transport is backwards, i.e., fluid transport is from blood to CSF, and the primary driving force for fluid movement is active Na^+ transport. Na^+ first enters the epithelium across the basolateral membrane down its electrochemical potential gradient, and then is pumped out across the ventricular membrane into the CSF.

Active Na^+ transport is followed by the transport of HCO_3^-, Cl^- and H_2O into the CSF, and this is accompanied by a small net absorption of K^+ from CSF to blood. Na/K pumps in the ventricular membrane account for both Na^+ and K^+ transport across the plexus.

The choroid plexus and the retinal pigment epithelium (Miller, Steinberg & Oakely, 1978) are unique in that the Na^+ pumps are on the brush border membrane; in most other epithelia the pumps are found on the basolateral membrane (*see* DiBona & Mills, 1979).

The fact that the choroid plexus Na/K pumps are readily accessible on the brush border membrane has allowed us to study the mechanism of active ion transport in epithelia more closely than can be done with such tissues as the gallbladder. The first part of the paper describes radioactive tracer experiments. The second part described electrophysiological experiments using both potential and K^+-sensitive microelectrodes. All electrophysiological measurements are interpreted in terms of a computerized nonsteady-state model (Lew, Ferreria & Moura, 1979). In the choroid plexus the model can be made analytical by utilizing measured values. Preliminary reports have been presented (Wright, 1977, 1978*a*, *b*; Zeuthen & Wright, 1978; Zeuthen, 1979).

These studies relate to the mechanism of CSF production and the regulation of the K^+ concentration in the extracellular fluids of the brain. In addition, our conclusions should be of general interest because they bear on mechanisms of active ion transport in epithelia where the Na/K pumps are on the basolateral membranes.

Part 1: Tracer Experiments

Materials and Methods

Transport Experiments

The unidirectional influx of K^+ across the ventricular surface of the Bullfrog choroid plexus was measured as described previously for anions (Wright, 1974[1]). Each plexus was mounted in a Lucite chamber (Fig. 1, Wright, 1974) that permitted the ventricular surface of the plexus to be exposed for brief periods (0.5 to 4 min) to a Ringer's solution containing ^{42}K and ^{3}H-mannitol. At the end of the incubation period the tissue was punched from the chamber, rinsed quickly in "cold" Ringer's solution, dissolved in tissue solubilizer, and assayed for radioactivity using liquid scintillation counting. Mannitol was included in the test solutions as an extracellular marker, and it completely equilibrated with the ventricular spaced within 0.5 min (*see* Fig. 9, Wright, 1974). The total serosal area of the plexus was 12–15 mm^2, and when mounted in the flux chamber the area of the exposed surface was 6.2 mm^2. In a series of five experiments where the average weight of the whole plexus was 4.2 ± 0.2 mg, the tissue water accounted for $81 \pm 4\%$ of the weight and $53 \pm 6\%$ of the water was extracellular (*see also* Wright, 1972*a*).

The efflux of K^+ from the epithelium was measured by mounting the plexus in the same Lucite flux chamber. In this case the tissue was loaded with ^{42}K from the ventricular surface of the tissue in the absence of serosal fluid, and, after equilibration had been reached, the rate of ^{42}K efflux from the plexus into the CSF was measured. This was accomplished by removing the saline containing the ^{42}K from the ventricular surface of the tissue, rinsing the chamber and the surface of the tissue free of isotope, and perfusing the CSF compartment with ^{42}K-free saline. The volume

[1] No corrections were made for the presence of unstirred layers at the ventricular surface of the plexus, 120 μm, and so the transport parameters refer to the brush border membrane and the adjacent unstirred layer.

of the saline on the ventricular surface was 200 µl, and this was replaced at a rate of 4 ml/min using a multichannel perfusion pump (Sage, Model # 375a). One channel was used to deliver saline to the chamber via a glass micropipette, and a second channel was used to remove saline through a micropipette at the same rate. A fraction collector (ISCC Model # 1200) was used to collect the effluent at 1-min intervals. At the termination of the experiment the plexus was removed from the chamber and the remaining ^{42}K in the tissue was determined as described above. Each fraction was assayed for ^{42}K and, after correction for decay, the amount of ^{42}K in the tissue was estimated at 1-min intervals throughout the experiment. The results are plotted as the log of the percentage of the initial amount of ^{42}K remaining in the tissue against time. In these experiments the tissue was mounted on a Millipore filter (0.22 µm pore diameter), and the volume of the serosal fluid was 4 ml.

Tissue K^+

The amount of K^+ in the choroid plexus was estimated using a lithium internal standard flame photometer (Instrumentation Laboratory Inc., Boston, Mass., Model 143). Ions were extracted from the tissue by shaking them in 0.5 ml of 1 N HNO_3 for 24 hr. Lithium chloride was added to the extract to give a final concentration of 15 mM, and the K levels were read on the flame photometer against standard solutions.

Ouabain Binding

The binding of the cardiac glycoside to the ventricular surface of the choroid plexus epithelium was measured using ^3H-ouabain (Quinton, Wright & Tormey, 1973). Plexuses were incubated for 1–150 min in solutions containing 0.1–10 µCi/ml of ^3H-ouabain and 1×10^{-9} to 1×10^{-4} M ouabain. After the incubation period the tissues were washed with ouabain free Ringer's solution to remove free extracellular ^3H-ouabain. Less than 4% of the bound ouabain was lost per hour when tissues were incubated in ouabain-free solutions. Each plexus was then dissolved in NCS tissue solubilizer and assayed for tritium using liquid scintillation counting.

Solutions

The majority of the experiments were carried out at 22 °C in a bicarbonate Ringer's solution containing (in mM): 85, NaCl; 2, KCl; 1, $CaCl_2$; 1, $MgSO_4$; and 25, $NaHCO_3$ gassed with 5% CO_2/95% O_2. In the experiments where the K^+ concentration was varied between 0.3 and 4 mM, the chloride was replaced with isethionate in an attempt to minimize problems with changes in the $[K^+]$ $[Cl^-]$ product. Some experiments were carried out in phosphate-buffered Ringer's solution containing (in mM): 105.4, NaCl; 2, KCl; 1, $CaCl_2$; 1, $MgSO_4$; and 2.5, $NaHPO_4/NaH_2PO_4$ (pH 7.3) and gassed with 100% oxygen. In one series of experiments NaCl was replaced completely with choline chloride, and in another series the NaCl partially replaced with $NaHCO_3$. Solutions were equilibrated with the appropriate CO_2/O_2 gas mixture to maintain the pH at 7.3.

Radioactive isotopes (^{42}K, ^3H-mannitol, ^{14}C-mannitol, ^3H-ouabain, and ^{14}C-sucrose) were obtained from New England Nuclear Corp., Boston, Mass. Radioactive samples were assayed using a Beckman LS 250 Scintillation Counter. In double label experiments the effect of spillover from ^{14}C and ^{42}K into the ^3H channel was minimized by arranging it so that the counting rate in the ^3H channel was at least ten times higher than the other channels. All samples were corrected, where appropriate, for background, quenching, spillover, and radioactive decay. All tracer fluxes, moles cm^{-2} hr^{-1}, are expressed in terms of the window area of the flux chambers (6.2 mm^2).

List of Symbols

K_i^+	The apparent potassium concentration in compartment *i*.
Na_i^+	The apparent sodium concentration in compartment *i*.
Cl_i^+	The apparent chloride concentration in compartment *i*.
v	Index: ventricular compartment.
c	Index: cellular compartment.
s	Index: serosal (or blood) compartment.
P_k^{ij}	The passive permeability per unit area of epithelium of ion *k* of the membrane separating compartment *i* and *j*.
$\bar{P}_k$	Total permeability of the cell to ion *k*.
Pk^{ij}	The active flux of ion *k* into compartment *j* from compartment *i*.
h	Cell height or rather volume to surface ratio of cells.
r	The coupling-ratio between active K^+ and Na^+-fluxes caused by a pump.
Z_i	Valency of ion *i*.
RT/F	25.3 mV at room temperature.
E^{ij}	The electrical potential difference between compartment *j* and *i*: $E^{ij}=E^j-E^i$.
E_k	The electrochemical potential of ion *k*.
i_k^+	The current carried into the cell by ion k^+.
g_K^-	The partial conductance for, in this case, K^+.
J	The potassium flux, e.g., J^{uc}=undirectional K^+ flux from ventricle to cell.

Results

A. K^+ Fluxes

1) Time course of ^{42}K uptake across the ventricular surface. In control experiments the ^{42}K influx into the choroidal epithelium across the brush border membrane was linear for at least 2 min and the slope corresponds to 2.85 µmol cm^{-2} hr^{-1} (Wright, 1978*b*, Fig. 9). At four minutes the uptake was nonlinear, and this is due to the increase in the specific activity of K^+ inside the cell and the resultant backflux of isotope out of the cell, probably, across the apical membrane. It should be noted that ^3H-mannitol, used to correct for the amount of extracellular ^{42}K in the fluid adhering to the ventricular surface of the plexus, reached a steady-state within 0.5 min and this amounted to 1.5 µmol/plexus, i.e., at 0.5 min the extracellular ^{42}K was about equal to that in the tissue, but that after 2 min this decreased to less than 25% of the total in the tissue. Since the K^+ diffusion coefficient is about a factor of three greater than that of mannitol, 1.9×10^{-5} *vs.* 0.67×10^{-5} cm^{-2} sec^{-1}, ^{42}K must equilibrate with the extracellular spaces in less than 10 sec.

In the presence of 1×10^{-4} M ouabain the ^{42}K influx was linear for at least 4 min and the slope corresponded to 0.44 µmol cm^{-2} hr^{-1}, i.e. ouabain produced a 85% inhibition of the K^+ influx (Wright, 1978*b*, Fig. 9: *see also* Fig. 2 and Table 1).

Similar experiments were carried out from the vascular side of the plexus, but owing to the presence

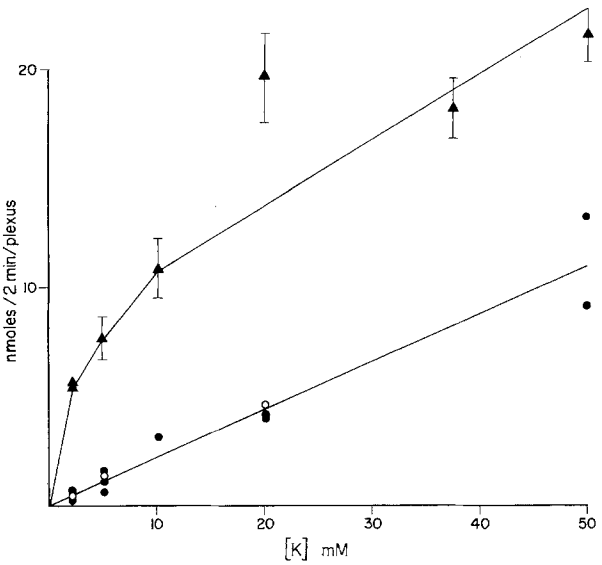

Fig. 2. Kinetics of ^{42}K uptake. Fluxes were obtained from 2-min uptakes from the ventricular solution and the results, expressed as nmol/plexus, are plotted against the potassium concentration. In control experiments (▲) the tissues were preincubated with 2 mM K saline and they were exposed to high K concentrations only during the flux period. Some tissues were exposed to 1×10^{-3} M ouabain for the 30 sec prior to the flux determination (●), and others were preincubated for 3 hr in K-free saline containing 1×10^{-3} M ouabain at 0 °C (○). Note that exposing the tissue to ouabain for 30 sec or to K-free solutions and ouabain eliminated the saturable uptake of potassium. In these experiments the K concentration was varied by replacing NaCl with KCl. Control experiments were carried out with five plexuses at each concentration and all other data points are single experiments. In the presence of ouabain the slope was 0.22 nmol/mM ($r=0.97$) and in the absence of ouabain the slope was 0.28 nmol/mM ($r=0.80$) over the concentration range 5–50 mM. The serosal area of the plexus was 6.2 mm^2

of nonepithelial tissue (blood vessels and connective tissue, Fig. 1) these gave a less-than-satisfactory estimate of the influx across the basolateral surface of the epithelium. However, the rate of K^+ uptake was 0.40 μmol cm^{-2} hr^{-1}, and ouabain (1×10^{-4} M) reduced the rate to 0.24 μmol cm^{-2} hr^{-1}, i.e., the ouabain-sensitive influx into the tissue across the serosal surface was 0.16 μmol cm^{-2} hr^{-1}. This rate of active transport was less than 6% of that across the brush border membrane. Since $^{42}K^+$ uptake across the serosal surface must also include transport into the nonepithelial cells of the plexus, we conclude that active $^{42}K^+$ transport into the epithelial cells is essentially limited to the brush border membrane. This agrees with ^{3}H-ouabain binding studies (Quinton et al., 1973) which show no binding to the basolateral membrane of the epithelium.

2) Kinetics of K^+ uptake. The rate of ^{42}K uptake into the plexus across the brush border membrane was first measured over a potassium concentration range of 2–50 mM (Fig. 2). In the control experiments the influx consisted of both diffusional and saturable components. However, when the tissue was either exposed to ouabain (1×10^{-3} M) for 30 sec immediately prior to the influx measurement or depleted of K_c^+ and poisoned with ouabain, the saturable component was eliminated and the influx was a linear function of concentration over the entire concentration range. The slopes of the diffusional fluxes in all three experiments were not significantly different from each other. Subtracting the diffusional component from the control curve yields a curve with a K_m of 1.5 mM and a V_{max} 8 μmol cm^{-2} hr^{-1}.

In order to avoid problems about the uncertainties of K^+ in unstirred layers (Fig. 8) a more detailed analysis of the kinetics of K^+ transport was conducted in the following manner. First, tissues were incubated for 12 hr in K^+-free saline at 0 °C to leach out the K_c^+. The tissues were then mounted in the flux chambers, allowed to equilibrate with K^+-free saline at 22 °C, and then exposed for 2 min on the ventricular surface to saline containing ^{42}K, K^+ and ^{3}H-mannitol. It should be noted that in these experiments the change in K^+ concentration due to addition of the isotope was taken into account, and, to minimize any cell swelling due to variations in the $[K^+][Cl^-]$ product (*see also* footnote 2, p. 114), the chloride salts in the saline were replaced with isethionate salts. Control experiments showed no significant effect of isethionate on the rate of K^+ transport.

In many cells the relationship between K^+ pumping and K^+ concentration is not adequately described by Michaelis-Menten kinetics, but by a sigmoid curve (*see* Schwartz, Lindenmayer & Allen, 1975; Glynn & Kalish, 1975). A more appropriate kinetic model is one in which there are multiple sites for the ligands on the carrier. For example

$$J = \frac{J_{max}}{(1 + K_m/K^+)^n}$$

where n is the number of binding sites on the carrier for K^+. Plots of $[K^+]/J^{1/n}$ against $[K^+]$ for K^+ fluxes in the choroid plexus are shown in Fig. 3. Estimates of J_{max} and K_m are obtained from the slopes of the curves ($J_{max}^{-1/n}$) and the intercepts on the horizontal axis, respectively (*see* Garay & Garrahan, 1973). In the case of a one-site model ($n=1$) the data points at the low concentrations deviate above the regression line, but a better fit is obtained for a two-site ($n=2$) model. The regression coefficient is 0.99 for $n=2$, but only 0.93 for $n=1$. This suggests that the choroid plexus pump has two binding sites for K^+ on the outside of the ventricular membrane. The K_m's and V_{max} for the two site model are 0.3 mM and 8 μmol cm^{-2} hr^{-1}.

3) Membrane potentials and K^+ transport. The data in Fig. 2 may also be used to explore the effect of potential on the K^+ transport. In control experiments the electrical potential across the ventricular membrane E^{vc} was -47 mV with $K_v^+ = 2$ mM (Table 5), and when K_v^+ was increased to 50 mM (Fig. 12) the potential depolarized by about 10 mV. Brief exposure of the plexus to 1×10^{-3} M ouabain also reduced the membrane PD 10 mV (Fig. 8) and variation of the K_v^+ under these circumstances depolarized E^{vc} by 0.13 mV for each mV change in E_K^{vc}. Finally, leaching out the intracellular K and poisoning the pump with ouabain reduced the control PD to -10 mV (Fig. 2, Zeuthen & Wright, 1978).

Under these experimental conditions, Fig. 2, the passive K^+ uptake, i.e., the nonsaturable component, was independent of E^{vc} and the fluxes correspond to a permeability coefficient of 3×10^{-5} cm sec^{-1}. At a K^+ concentration of 2 mM this gives a passive uptake of 0.2 μmol cm^{-2} hr^{-1}. Since the passive unidirectional flux of K^+ across the epithelium, J_K^{vs} amounts to 0.09 μmol cm^{-2} hr^{-1} (Wright, 1978*b*), about half the passive unidirectional uptake must occur through the tight junctions and the remainder through the brush border membrane. A passive influx of ~0.1 μmol cm^{-2} hr^{-1} is close to that predicted from the flux ratio equation, $(J^{vc}(\text{passive}) = J^{cv} \cdot \exp[(E^{vc} - E_K^{vc})zF/RT] \sim 0.25$ μmol cm^{-2} hr^{-1} since $E^{vc} = -35$ mV, $E_K^{vc} = -96$ mV (Table 5) and assuming that $J^{cv} \sim J^{vc}(\text{total}) - J^{vs}(\text{net}) \sim 2.86 - 0.03 \sim 2.83$ μmol cm^{-2} hr^{-1}). The apparent insensitivity of the K^+ permeability to the variation in $[K_v^+]$ is probably due to the fact that: (i) there is virtually no variation in the transepithelial membrane potential, E^{vs}, as $P_K/P_{Na} \sim 1$ (Wright, 1972*b*; and page 114); (ii) the high conductance of the tight junctions (pages 120 & 124); and (iii) the variation in the electrical driving force across the brush border membrane is relatively small, i.e., $[(E^{vc}F/RT)/1 - \exp(E^{vc}F/RT)]$ varies by less than 2 under these experimental conditions (Katz, 1966).

4) Effects of ouabain. Figure 2 shows that ouabain produces a dramatic reduction in the K^+ influx. Full inhibition of transport occurred at 5×10^{-6} M and 1×10^{-7} M caused 50% inhibition (Wright, 1978*b*, Fig. 11). This dose-response curve is very similar to that obtained earlier for active anion transport (Wright, 1978*c*; Fig. 3), the plexus Na/K ATPase (Wright, 1978*b*, Fig. 7), and ^{3}H-ouabain binding (Fig. 5).

5) Effects of other drugs on K^+ transport. Phloretin and ethacrynic acid are known to inhibit both Na/K-ATPase and Na/K pumps in a variety of cells and tissues (e.g., Robinson, 1969; Hase, Kobashi & Kobayashi, 1973). In the plexus both of these drugs inhibit active K^+ transport to about the same degree as ouabain (Table 1). Ouabain and ethacrynic acid together inhibit the K^+ influx to a greater degree than either alone, about 98%. The passive unidirectional K^+ fluxes across the epithelium, 0.09 μmol cm^{-2} hr^{-1} (Wright, 1978*b*), and the passive influx across the ventricular surface of the epithelium in the presence of ouabain and ethacrynic acid, 0.07 μmol cm^{-2} hr^{-1} (Table 1) are indistinguishable.

Amiloride, a blocker of sodium channels in some epithelia, reduces the K^+ influx 27%, and 1 mM ATP

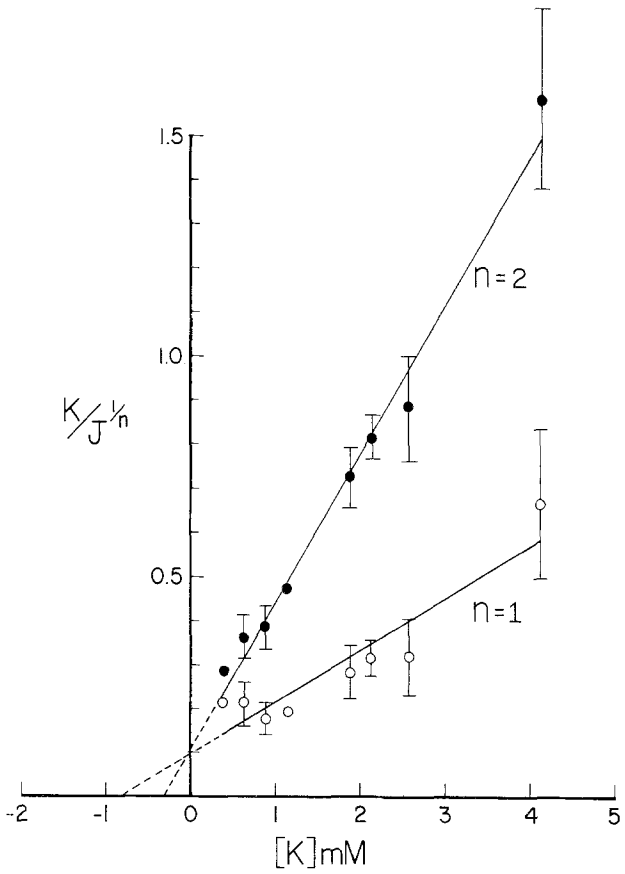

Fig. 3. The K influx across the ventricular surface of the choroid plexus as a function of K concentration. In these experiments the tissues were preincubated for 12 hr in K-free saline at 0 °C. The K influx (nmol/plexus/2 min) was measured at 22 °C at K concentrations ranging from 0.4 to 4.0 mM. NaCl in the saline was replaced with Na isethionate, and the K concentration was varied by adding K isethionate. Each point was the mean of three estimates and standard errors are indicated when the errors are larger than the points. The fluxes (J^{vc}) were plotted as $(K)/J^{1/n}$ against the potassium concentration (K), where n is the number of sites on a transport carrier. Regression lines are fitted for $n=1$ and $n=2$; and the regression coefficients were 0.93 and 0.99, respectively. It can be seen for $n=1$ that the data points for the lower K concentrations deviate upwards from the regression line, but this is largely eliminated in the case of the $n=2$ plot. The serosal area of the plexus was 6.2 mm^2

Table 1. Effect of drugs on K transport across the apical membrane

	Flux (μmol cm^{-2} hr^{-1})	% Change
Control	2.86±0.21 (7)	–
Ouabain (7×10^{-6} M)	0.47±0.08 (6)	−84%
Phloretin (5×10^{-4} M)	0.47±0.14 (4)	−83%
Ethacrynic acid (1×10^{-3} M)	0.80±0.09 (5)	−72%
Ethacrynic acid + ouabain	0.07±0.03 (2)	−98%
Amiloride (1×10^{-4} M)	2.10±0.23 (3)	−27%[a]
ATP (1×10^{-3} M)	4.13±9.53 (5)	+45%[a]

All experiments were carried out in HCO_3^- saline as described in the text. Drugs were added to the saline to give the final concentrations indicated, and the % change in flux is indicated.

[a] Both effects are statistically significant at the 5% level.

Table 2. Effect of ions on K transport across the apical membrane

	Flux (μmol cm^{-2} h^{-1})	% Change
Control	2.02±0.55 (3)	–
Choline Cl	0.69±0.04 (3)	−66%
Control	1.49±0.16 (17)	–
HCO_3	2.70±0.24 (13)	+81%

All experiments were carried out in phosphate saline. In the first series choline Cl replaced NaCl on a mole for mole basis, and in the second a HCO_3 concentration of 25 mM was obtained by substituting $NaHCO_3$ for NaCl. All experiments were carried out at pH 7.3.

stimulates the flux 45%. The origin of these latter effects is not clear.

6) The effect of other ions on the K^+ influx. The effect of HCO_3^- and Na^+ on the active transport of K^+ across the apical membrane is given in Table 2. HCO_3^- at a concentration of 25 mM doubles the rate of K^+ pumping, and this is consistent with earlier reports (Wright, 1977). The K_m for bicarbonate was 7.5 mM, and other lipid soluble buffers, e.g., glycodiazine, were able to mimic the HCO_3^- effect. This has been explained in terms of a model where the Na/K pump rate is limited by the influx of Na^+ into the epithelium across the basolateral membrane. Na^+ enters across the basolateral membrane in exchange for H^+, and H^+ is generated within the epithelium by the dissociation of weak acids such as H_2CO_3 that are able to enter the cell through non-ionic diffusion.

Preincubation of the plexus in sodium-free Ringer's solution (choline$^+$ substituted for Na^+) for 1 hr reduced the K^+ flux 66%. This suggests that intracellular sodium is required for normal operation of the K^+ pump, i.e., the Na^+ and K^+ pumps are coupled.

7) ^{42}K efflux across the ventricular membrane. Experiments were carried out to measure the efflux of ^{42}K from the epithelium (J_K^v) by loading the tissue with isotope and then monitoring the washout of ^{42}K into the ventricular compartment. The experiments were performed under similar conditions to those with K-sensitive microelectrodes (Fig. 9). The protocol was designed to minimize two potential problems, i.e., the influence of nonepithelial cells in the tissue; and recycling of ^{42}K across the brush border by the Na/K pump.

In the rat choroid plexus only 25–40% of the total number of cells in the tissue are epithelial cells (Quay, 1966). The nonepithelial cells include fibroblasts, endothelia, and erythrocytes which all face the serosal side of the epithelium (Fig. 1). To reduce the nonepithelial K^+ compartment, the serosal face of the plexus was exposed to 1×10^{-4} M ouabain for 60 min prior to loading. This reduced tissue K^+ by 50–60% (*see* below), but did not effect active transepithelial transport (Wright, 1972*b*, 1974). Autoradiographic studies (Quinton et al., 1973) show no ^3H-ouabain binding to the basolateral membrane of the epithelium.

To reduce recycling of ^{42}K across the brush border during the washout experiments, the ventricular face of the epithelium was exposed for 30 sec to 1×10^{-4} M ouabain. The treatment abolished the active K^+ influx into the epithelium across the brush border membrane (*see* Fig. 2).

In the experiment shown in Fig. 4 the plexus was first mounted in the flux chamber and the serosal face of the tissue was exposed to 1×10^{-4} M ouabain for 60 min. The epithelium was then loaded with ^{42}K from the ventricular surface by exposing the plexus to saline containing 2 mM K and ^{42}K for 1 hr. The "hot" ventricular solution was then removed and the surface of the epithelium was washed with saline containing 1×10^{-4} M ouabain for 30 sec. 200 µl of saline was placed in the ventricular compartment and this was perfused at a rate of 4 ml/min. Fractions of the effluent were collected at 1-min intervals, and at the termination of the experiment the plexus was removed from the chamber and was assayed for ^{42}K. The efflux was plotted as the log of the percentage of the initial counts remaining in the tissue against time.

The washout consisted of two phases – a fast and a slow exponential curve (Fig. 4). The fast exponential curve (*B*) was obtained by subtracting the slow exponential (*A*) from the total curve. The half times for the efflux curves were 3.5 and 19 min. In three experiments the rate constants for the fast phase was 0.197 ± 0.046 min^{-1} and for the slow phase 0.037 ± 0.004 min^{-1}.

Microelectrode experiments (Fig. 9) show that ouabain produces a two-phase drop in the intracellular K^+ activity in the choroidal epithelium. The half time for the fast phase was 2 min and for the slow phase 25 min. This suggests that the fast phase of

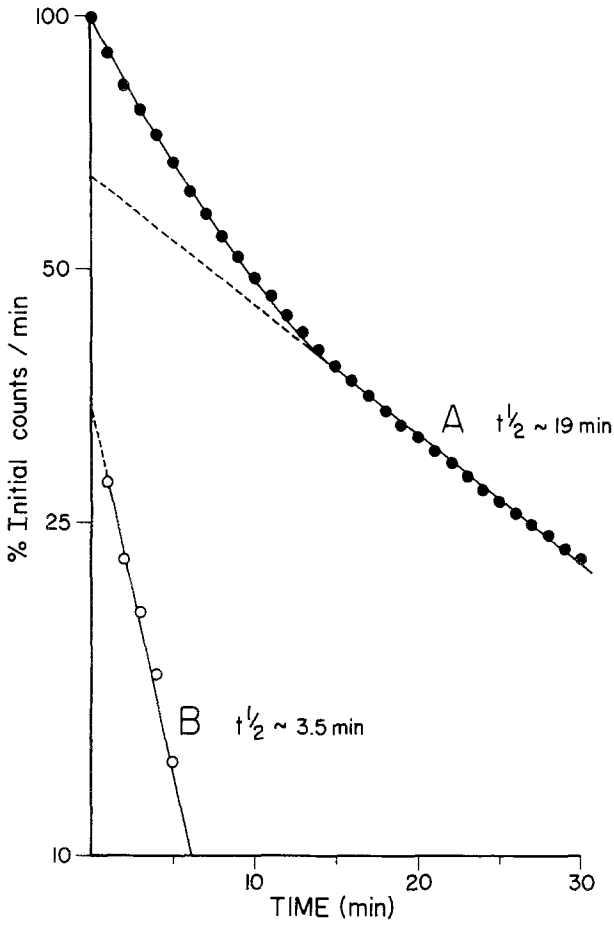

Fig. 4. The efflux of ^{42}K from the choroidal epithelium. The plexus was mounted in the flux chamber and then loaded with ^{42}K from the ventricular solution. The isotope was removed from the surface of the tissue and the plexus was exposed to 1×10^{-4} M ouabain for 30 sec. The ventricular solution was perfused at a rate of 4 ml/min and samples were collected at 1-min intervals. The results were plotted as the log of the % of the initial counts remaining in the tissue against time. The washout curve consisted of two phases – a fast and a slow phase. The fast phase (*B*) was obtained by subtracting the slow curve (*A*) from the total curve

the ^{42}K washout from the epithelium is due to the loss of cytoplasmic K^+ across the brush border membrane. The origin of the slow component (Figs. 4 and 9) is unclear, but it may be related to a slow efflux of noncytoplasmic K^+ (e.g., mitochondrial and/or nuclear) into the cytoplasm.

The fraction of ^{42}K in the fast and slow intracellular compartments of the epithelium in the loaded tissue may be estimated from the zero time intercepts of the efflux curves (*A* and *B*, Fig. 4) as described by Huxley (1960). The intercepts and the rate constants predict that 60% of the ^{42}K is in the fast compartment, i.e., $0.6 \times 15 \times 10^{-9}$ moles/plexus $= 9 \times 10^{-9}$ moles (*see* Table 3). The K^+ unidirectional efflux from the rapidly exchanging epithelial compartment into the CSF can be estimated from the rate constant and the amount of potassium in the pool, i.e.,

$$J^{cv} = k_{\text{fast}} \cdot \frac{(V \cdot C)}{A}$$

where J^{cv} is the unidirectional efflux, k_{fast} is the rate constant for the fast efflux, V is the volume of the pool, C is the concentration of K^+ in the pool and A the area of the chamber window. Since $V \cdot C = 9 \times 10^{-9}$ moles and $k_{\text{fast}} = 0.197\ \text{min}^{-1}$, the efflux amounts to 1.7 μmol $\text{cm}^{-2}\ \text{hr}^{-1}$. This value is close to that predicted from the net K^+ flux (J^{net} 0.03 μmol $\text{cm}^{-2}\ \text{hr}^{-1}$) and the unidirectional K^+ uptake from the CSF ($J^{vc} = 2.85$ μmol $\text{cm}^{-2}\ \text{hr}^{-1}$), i.e., $J^{cv} = J^{vc} - J^{\text{net}} = 2.82$ μmol $\text{cm}^{-2}\ \text{hr}^{-1}$.

B. Sodium and Potassium Contents

Plexuses incubated for $2^1/_2$ hr in the HCO_3 saline contained $2.4 \pm 0.2\ (5) \times 10^{-7}$ and $2.6 \pm 0.3\ (5) \times 10^{-7}$ moles of sodium and potassium, respectively (Wright, 1978*b*, Fig. 8). Using estimates of tissue water (81 ± 4 (5)% of the wet wt) and the mannitol spaces (53 ± 6 (5)% of the total water), these amounts yielded intracellular Na and K concentrations of 17 and 154 meq/liter.

In five experiments the steady-state specific activity of ^{42}K in the choroid plexus was determined. The plexuses were incubated in HCO_3^- saline containing ^{42}K for $2^1/_2$ hr, and at the end of the experiment the amount of K^+ in each tissue was estimated by flame photometry and by the ^{42}K content. The chemical determinations gave a value of $2.6 \pm 0.2 \times 10^{-7}$ moles and the ^{42}K content gave a value of $1.0 \pm 0.1 \times 10^{-7}$ moles, i.e., the specific activity of ^{42}K in the tissue at the steady-state was only 40% of that in the incubation medium. One other series of five similar experiments gave an intracellular specific activity 55% of the external solution. The results suggest that only 40–55% of the total K^+ in the tissue exchange with the external solutions. Similar observations have been reported for the intestine and urinary bladder (Nellans & Schultz, 1976; Robinson & Macknight, 1976).

The exchange of ^{42}K between the CSF and the plexus and the blood and the plexus was determined by mounting tissues in the flux chamber and exposing the tissue to ^{42}K in the CSF and/or the serosal compartments. Table 3 shows that ~25% of the ^{42}K space is accessible from the blood and ~75% from the CSF. Ouabain in the blood reduces the ^{42}K in the tissue by ~60% and ouabain in both the blood and CSF reduced the ^{42}K by ~90%. These results suggest that (i) about 40% of the ^{42}K pool in the total plexus, 15×19^{-9} moles, is within the epithelium,

Table 3. Distribution of intracellular ^{42}K in the choroid plexus

	^{42}K ($\times 10^{-9}$ moles/plexus)		
	^{42}K in blood	^{42}K in CSF	^{42}K in CSF and blood
No ouabain	9.3 (28%) 13.6	29.0 (61%) 20.3	49.3 (100%) 32.1
Ouabain in serosal fluid	1.2 1.6 (3%) 0.9	14.3 8.0 (23%) 5.8	10.7 (38%) 18.9
Ouabain in ventricular and serosal fluids	–	–	3.7 (9%) 3.5

Tissues were mounted in the flux chamber (area 6.2 mm^2) and ^{42}K, ^{3}H-mannitol, and ouabain were present in the CSF and/or blood compartments as indicated. The saline was buffered with bicarbonate and the tissues were incubated for $2^1/_2$ hr. The amount of intracellular ^{42}K in the tissue was estimated at the end of the experiments from the ^{42}K content and the ^{3}H-mannitol spaces. Each number refers to a single tissue, and the number in parenthesis the mean value for each experimental condition expressed as a percentage of the total ^{42}K in the tissue in the control experiment.

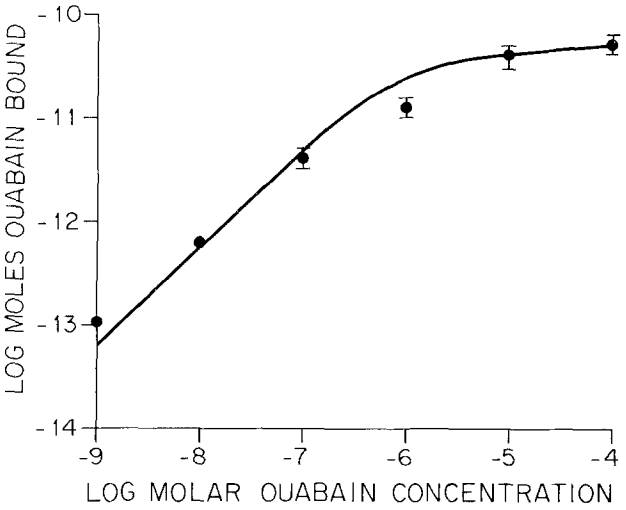

Fig. 5. Ouabain binding to the choroid plexus as a function of ouabain concentration. The log of the amount bound per plexus is plotted against the log of the ouabain concentration. Each point is the mean of 5–9 estimates with the SEM indicated whenever it is larger than the size of the symbol. The curve is given by an equation of the type $B=(B_{max}\times C)/(C\times K_m)$ with a B_{max} of 4.5×10^{-11} moles/plexus and a K_m of 8×10^{-7} M. The serosal area of the plexus was 14 mm^2

and (ii) the permeability of the basolateral surface of the epithelium is significantly less than the permeability of the ventricular membrane.

C. Ouabain Binding

Binding of ouabain to Na/K ATPases follows the mass law equation where the association reaction follows second-order kinetics and the dissociation reaction follows first-order kinetics, i.e.,

$$O+E\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}}OE$$

where O, E and OE represent ouabain, the enzyme, and the ouabain-enzyme complex, respectively, and k_1 and k_{-1} are the association and dissociation rate constants. In the steady-state the amount of ouabain bound to the enzyme (B) is given by the expression

$$B=\frac{B_{max}[O]}{[O]+K_m}$$

where B_{max} is the maximum bound, $[O]$ is the ouabain concentration and K_m is the ouabain concentration that produces $0.5\,B_{max}$. The K_m is also described by the ratio of the dissociation and association rate constants ($K_m=k_{-1}/k_1$). The time course of ouabain binding at any given concentration is given by $B_t/B=(1-\exp-kt)$ where B_t is the amount bound at time t and k is a rate constant related to k_1 and k_{-1} by the expression $k=k_1[O]+k_{-1}$. The association rate constant is given by the expression,

$$k_1=\frac{Bk}{B_{max}[O]}.$$

Figure 5 shows the relationship between ouabain binding (B) and $[O]$ over the range 1×10^{-9} to 1×10^{-4} M. The experimental points were fitted by a curve with a B_{max} and K_m of 4.5×10^{-11} moles and 8×10^{-7} M, respectively. The time course of binding at four different ouabain concentration curves is given in Fig. 6. The half time ($T^{\frac{1}{2}}$) for binding decreased from 23 min at 4×10^{-7} M to about 1 min at 1×10^{-4} M. A half time of 23 min corresponds to a rate constant of 3×10^{-2} min^{-1}, and substitution of this and the other parameter into the equation above gives a value of 3.8×10^4 $moles^{-1}\,min^{-1}$ for k_1 $\left(k_1=\frac{2.25\times10^{-11}\times3\times10^{-2}}{4.5\times10^{-11}\times4\times10^{-7}}=3.8\times10^4\ moles^{-1}\ min^{-1}\right)$. Using k_1 and K_m (8×10^{-7} M), value of 3×10^{-2} min^{-1} is obtained for k_{-1}. The values obtained for K_m, k_1, k_{-1} are intermediate between those reported for human erythrocytes (Erdmann and Hasse, 1975) and rabbit renal tubule (Shaver and Stirling, 1978) at 22–25 °C (see also Harms & Wright, 1980).

Using the values estimated for the kinetics of ouabain binding to the choroid plexus, it is possible to calculate the half time for ouabain binding at any

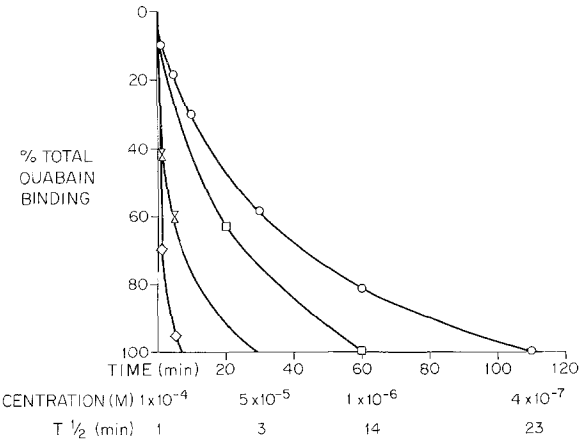

Fig. 6. Time course of ouabain binding to the choroid plexus as a function of concentration. The amount bound is plotted as a percentage of the final amount bound at each concentration (*see* Fig. 8). The half time ($T^1/_2$) is the time required for the plexus to bind 50% of the final amount

other concentration. At 1×10^{-4} M the kinetic parameters predict a half time for ouabain binding of 10 sec. This is considerably faster than that shown in Fig. 6, but it is consistent with the observation that 1×10^{-4} ouabain eliminates the electrogenic component of the membrane potential in 7 sec (Fig. 8). Ten seconds after applying the glycoside 2.25×10^{-11} moles of ouabain are bound to the plexus, and this is sufficient to block K^+ transport 75% (*see* Fig. 5 and Wright, 1978*b*, Fig. 11).

As shown previously (Quinton et al., 1973), ouabain binding to the frog choroid plexus is limited to the ventricular membrane of the epithelium, and binding is specific as judged by the effects of K^+ and Mg^{++}. To confirm the specificity, we tested the effect of different glycosides on ^{3}H-ouabain binding. Plexuses were exposed to tritiated ouabain (5×10^{-7} M) and to solutions containing both ^{3}H-ouabain and 5×10^{-6} M ouabain, cymarin, or gitoxigenin. Cymarin inhibited ^{3}H-ouabain binding 94 ± 1 (3)%; ouabain inhibited 76 ± 1 (3)%, and gitoxigenin inhibited 62 ± 1 (3)%. The inhibitor constants (K_i's) for these glycosides on brain Na/K ATPases were cymarin 0.3 μM, ouabain 1 μM, and gitoxigenin 2 μM (Wilson, Sivitz and Hanna, 1970), i.e. the ranking of the effects on Na/K ATPases and ^{3}H-ouabain binding were identical.

Part 2: Electrophysiology

Materials and Methods

Microelectrodes and Recording

The microelectrode techniques employed for recording intracellular electrical potentials and ion activities were those described earlier (Zeuthen, Hiam & Silver, 1974; Zeuthen, 1978, 1980). The electrodes had a double barrelled tip with a total diameter of less than 0.3 μm. The reference barrel was pulled from a glass with an outer diameter of 1 mm, and inner diameter 0.5 mm and contained an internal fiber for easy filling. It had an impedance of 50–200 MΩ when filled with 2 M KCl and measured in physiological saline. The accompanying barrel, designed to contain the ion selective membrane, was pulled from a glass with an outer diameter of 1.5 mm and an inner diameter of 1.0 mm. When filled with 2 M KCl it had an impedance of 20–100 MΩ. By choosing tubes of different diameters for the two barrels one could minimize the outflow of reference solution into the cell (Nelson, Ehrenfeld & Lindeman, 1979) and maximize the signal-to-noise ratio of the ion-selective barrel. The ion-selective barrel had a sensitivity of 50–55 mV/10-fold change in K^+-activity, and response time of less than 1 sec. If the electrodes had finer tips than described above, the sensitivity decreased. The results are presented as apparent concentrations; the potassium is compared directly to an external reference solution. Thus an intracellular apparent K concentration of 90 mM is the true concentration, if the intracellular activity coefficients were similar to those of frog saline (about 0.76).

The intracellular electrical potential (E^{vc}) was recorded in two ways; either with single-barrelled micropipettes (20–100 MΩ) or with the reference barrel of the double-barrelled ion-selective electrodes. Using the single-barrelled electrodes, the potential was monitored by means of a fast negative capacitance recorder (WPI 701, WP Instruments, Inc., time constant of ~50 μsec) and E^{vc} was defined as the maximum electrical potential obtained during penetration. The electrical potential obtained with the reference barrel of the double-barrelled electrodes was displayed on a chart recorder (time constant ~0.1 sec), and E^{vc}'s obtained with double-barrelled electrodes were defined as those potentials which were constant within 5 mV over a period of 2–20 min.

Chamber and the Monitoring of Potentials

Choroid plexuses were mounted in an Ussing chamber with a window area of 6.2 mm^2 and supported by a millipore filter as described by Wright (1974), i.e., identical to that in Part I. The volume of the ventricular solution was between 20 and 400 μl and was renewed every 1–3 sec by continuous flow. The volume of the serosal solution was about 3 ml. The reference electrodes were Ag/AgCl which made contact with the ventricular and the serosal solution via 2 M KCl agar bridges; the serosal bridge which consisted of a PVC-tube of a diameter of 2 mm was placed in contact with the millipore filter. The ventricular bridge which consisted of a glass pipette of diameter 200–500 μm was placed 100–500 μm from the epithelium; this electrode was usually connected to system ground.

Current was passed across the tissue between two Ag/AgCl electrodes arranged symmetrically around the vertical axis of the chamber. The mucosal current passing electrode was made from a silver wire (diameter 1 mm) shaped into a ring of diameter of 3 mm. The electrode was placed 3 mm above the tissue. The serosal current passing electrode was a spiral of silver wire 0.5 mm in diameter and 30 cm long.

The distribution of electrical potential around and in the tissue was measured by microelectrodes. The potential of the ventricular solution E^v was measured with the tip of the electrode positioned within 50 μm of the apical membrane. The intracellular potential E^c was recorded with the tip inside the cell and the potential right behind the cells (the serosal potential E^s) was recorded with the microelectrode tip positioned within 10 μm of the basolateral membrane of the epithelial cells. The ratio between the voltages induced across the apical and serosal membrane, the voltage divider ratio ($\Delta E^s - \Delta E^c / \Delta E^c - \Delta E^v$), as well as the transepithelial impedance ($\Delta E^s - E^v / I$) could be calculated from the electrical potentials evoked in the tissue by a transmural current.

Solutions

Tissues were bathed at the ventricular and the blood side by a saline containing (in mM) 85 NaCl, 2 KCl, 1 $MgSO_4$, 1 $CaCl_2$ and 25 $NaHCO_3$ in equilibrium with 95% O_2/5% CO_2, pH 7.3. When the K^+ activity was increased in the ventricular solution Na^- was reduced to maintain isotonicity; thus if the K^+-concentration was 50 mM the Na^+-concentration was 60 mM.[2] The solutions were passed via peristaltic pumps (0.4–24 ml min^{-1}) into the ventricular side of the chamber, which was drained by gravity. The change of the perfusion solution was performed by a two-way syringe switch which was connected to the chamber via a thin tube. The dead volume between the switch and the bath was about 30 µl.

Results

Site of penetration. The choroidal epithelium is very folded, with several rims and folds branching off the central rim (*see* Nelson & Wright, 1974, plate 1). The percentage of successful penetrations was largest on the central rim. Furthermore, the epithelium at this central rim was flat over an area of 100 by 300 µm and perpendicular to the symmetry axis of the chamber. All results were derived from this part of the tissue and are expressed per cm^2 of epithelial cells.

A. The Intracellular Electrical Potential E^{vc}

When a single-barrelled electrode was advanced across the apical membrane the electrical potential increased abruptly at a rate of about 10–30 mV $msec^{-1}$, plateaued at a value between −30 and −60 mV, and subsequently depolarized. The plateau was maintained for between 1 msec and several minutes. In about 10% of the recordings the potential plateaued at about −15 mV for about 2–4 msec before increasing to the maximum plateau value. The distribution of maximal values obtained with five single-barrelled electrodes with impedances between 20, and 100 MΩ had a mean value of −46.1 mV ± 1.94 (49 cells in three animals). The reference barrel of the double-barrelled electrodes recorded an intracellular electrical potential of −45.2 mV ± 2.1 (34 cells in 13 animals), using the criteria that the potential should be constant within 5 mV over a period of at least 2 min. In one animal the maximal potential during penetration was compared, using either a double-barrelled electrode of an impedance of the reference barrel of 60 MΩ or a single-barrelled electrode of impedance 50 MΩ. The double-barrelled electrode recorded −43.1 mV ± 4.1 (13 cells) and the single-barrelled −46.7 mV ± 3.1 (25 cells); the difference between the two numbers is not significant. Therefore, double-barrelled electrode records a potential which is not smaller than those recorded by the finest single-barrelled electrodes.

B. The Transepithelial Potential Difference and Impedance

The transmural electrical potential difference was less than 2 mV and was constant within 2 mV even when K_v^+ was raised (in exchange for Na^+) as high as 112 mM (Fig. 12). This is within the accuracy achieved by the electrodes (*see* Materials and Methods, and Zeuthen, 1978). These results agree with Wright (1972*b*), who found that there was no or little transepithelial selectivity between Na^+ and K^+: $P_K/P_{Na} \simeq 1$.

In order to determine the impedance of the epithelium one must know (i) the current density at the flat part of the epithelium and (ii) the potentials evoked at each side of the epithelium itself.

The current crossing the flat part of the epithelium was determined by advancing the microelectrode through the ventricular solution towards the epithelium in 50 µm steps and recording the change in the potential evoked by the transmural current as a function of distance from the epithelium (Fig. 7). A current density of 1.04 mA ± 0.09 ($n = 17$, three animals) cm^{-2} of epithelium was obtained with a current of 200 µA across the whole tissue. The current density was uniform within 10% over the flat part of the epithelium.

The evoked voltage across the epithelial cell layer was recorded as the difference between the evoked potential measured with the microelectrode positioned immediately in front of the ventricular membrane (Fig. 7) and the evoked potential recorded immediately behind the epithelial cell layer. The tip of the microelectrode was estimated to be positioned behind the cell layer when the preceding advance (10 µm) had caused the recorded electrical potential abruptly to return from negative to near zero values. Ionophoretic ejection of dyes from the microelectrode has shown that this conclusion is correct in the rabbit intestine (T. Zeuthen, *unpublished*).

The impedance of the flat epithelium without its underlying connective tissues was determined as 24 Ωcm² for currents from ventricle to blood. When the current was in the other direction, from blood to ventricle, the measured impedance was always larger by a factor of 1.2 (*see* Table 4)[4]. The impedance of each epithelium was independent of the current; current densities up to 3.21 mA per cm^2. The varia-

[2] One may argue that in order to minimize changes in cell volume when extracellular K is replaced by Na, the product of the extracellular Cl activity and K activity should remain constant. This notion, however, only applies to cells where K^+ and Cl^- are distributed in equilibrium across the cell membrane as in muscle. In epithelia K^+ and Cl^- are not in equilibrium. In electrophysiological experiments we chose to reduce the period in which the tissue was exposed to osmotic imbalances (i.e., high K_o).

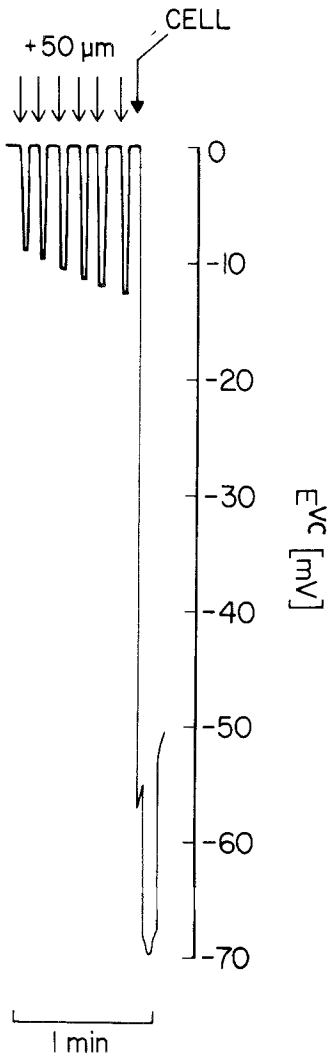

Fig. 7. The electrical potential evoked in the ventricular solution by a transmurally applied current of 200 μA as a function of distance from the ventricular membrane. Initially the tip of the electrode was about 300 μm from the ventricular membrane and the transmural current was applied for 3 sec causing an evoked potential of about −10 mV. The transmural current was applied at each step of 50 μm towards the epithelium until the tip penetrated the cell (in this particular penetration the cell depolarized fast as a result of the penetration). From the change in evoked potential as a function of the position, the current density into the epithelium could be calculated as 1.04 mA cm^{-2}. The scanning was performed vertically at the flat, horizontal part of the choroidal epithelium

tion was larger between animals than within each tissue.

The impedance of the whole tissue (epithelium plus underlying capillaries and connective tissues) was 128 Ωcm^2 when expressed in terms of the area of the window between the half chambers.

C. The Relative Resistances of the Apical and Serosal Membranes

By comparing the intracellularly evoked potentials with those evoked at the ventricular side and the serosal side of the epithelium, the resistance of the serosal membrane could be compared to that of the apical membrane. For currents (1.04 mA per true cm^2 of epithelium) passed from ventricle to blood (positive direction), the serosal membrane had a resistance which was nine times larger than that of the apical membrane (Table 4). For currents in the negative direction, the factor was six. These ratios were similar at current densities of 2.13 mA cm^{-2}.

Table 4. Electrical parameters

Animal No.	E^{vc} (mV)	$R_{ep}^{\rightarrow}$ (Ωcm^2)	$R_{ep}^{\leftarrow}$ (Ωcm^2)	$\alpha^{\rightarrow}$	$\alpha^{\leftarrow}$	R_{WT} (Ωcm^2)
9	−43	16.1	–	3.1	–	109
	±3.8	±1.0	–	±0.68	–	–
	(14)	(14)	–	(14)	–	–
10	47	39.0	45	4.4	5.95	159
	±1.4	–	–	±0.66	±0.66	–
	(12)	(2)	(2)	(12)	(6)	–
11*a*	−51	14.4	15.4	10.3	7.1	104
	±2.2	–	–	±1.3	±0.52	–
	(8)	(2)	(2)	(10)	(6)	–
11*b*	−49	33.1	46.8	9.7	7.7	–
	±1.8	±5.2	±7.6	±2.2	±1.73	
	(14)	(8)	(5)	(41)	(10)	–
12	−53	4.8	5.5	25	5.6	130
	(1)	(1)	(1)	(1)	(1)	–
14	−46	36.8	39.5	3.6	2.9	140
	±1.7	±4.2	±4.9	±0.54	±0.63	–
	(7)	(5)	(4)	(12)	(11)	–
Average	−48.1	24.0	30.4	9.4	5.8	128

An arrow pointing right is indicative of a current passing from the ventricle into the serosal compartment. R_{ep} is the impedance of the epithelium, R_{WT} the impedance of the whole tissue. α is the ratio of the resistance of the serosal membrane to the ventricular one. E^{vc} is the intracellular potential.

D. The Intracellular Chemical Potential E_K (K_c^+-Activity)

The chemical potential for potassium was recorded as 97.2 mV ± 1.8 (34 cells in 13 animals) relative to the chemical potential of the ventricular solution. As K_v^+ was 2 mM the apparent concentration of K^+ inside the cells was 90 mM. If we assume an activity coefficient of 0.76 K_c^+-activity becomes 67.5 mM.

1. The effects of cardiac glycosides. When the perfusion solution was changed to one containing ouabain (10^{-4} M) E^{vc} began to depolarize within 3 sec (Fig. 8), the time it takes to change the solution in the ventricular bath (*see* Materials and Methods). It first depolarized abruptly (2.4 mV sec^{-1}) by 10.2 ± 2.2 ($n=5$) (*see also* Zeuthen & Wright, 1978) and then decreased at a slower rate 6 mV min^{-1} for about 10 min to

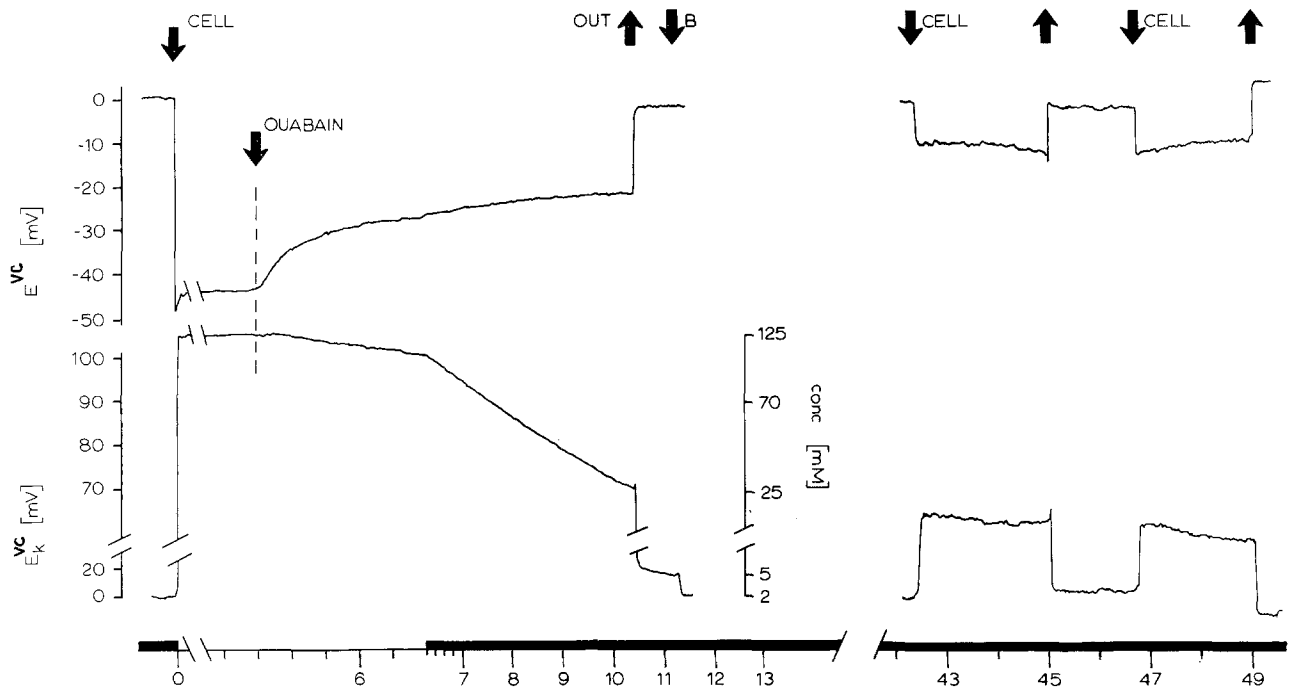

Fig. 8. The effects of ouabain (10^{-4} M) on the intracellular electrical potential E^{vc}, the intracellular chemical potential E_K for K^+, and on the apparent intracellular K^+ concentration (mM). All potentials are relative to the ventricular solution. The electrode was advanced into the *cell* at the first arrow. After 5 min and 30 sec ouabain was added to the ventricular solution. This caused E^{vc} to depolarize about 10 mV and E_K to decrease linearly with time. When the electrode was removed from the cell at *out* an unstirred layer was observed. It contained 5 mM of K^+ adjacent to the cells and was dissipated over a distance of about 300 μm, at *B*. At times 43 and 47 min two other cells were penetrated. The apparent intracellular K^+ concentration was now about 10 mM and decreased much slower than immediately after ouabain application

−12 mV where it remained virtually constant (Figs. 8 and 9). The chemical potential difference (E_K) began to decrease 5.5 sec later than the onset of the change in E^{vc} (Fig. 8). E_K fell linearly for the first 10 min after the application of the ouabain, by 0.11 mV sec^{-1}, and a halftime of about 9 min corresponding to a halftime in apparent concentration of 3 min (*see* Fig. 9). After it had decreased from 100 to 50 mV (equivalent of a drop in concentration from 100 to 10 mM) it fell at a slower rate with a halftime of about 50 min (Fig. 9).

Thus the initial abrupt depolarization in E^{vc} was complete in about 4 sec. If one allows for the 3 sec it took to change to the ouabain-containing perfusion solution, the action of the ouabain on the electrical parameter was complete in less than 7 sec. The speed and magnitude of the response clearly indicate that the pump is electrogenic.

The time course of the decline in E^{vc}_K and E^{vc} was the same whether it was determined from one cell or from multiple penetrations into separate cells. This excludes the possibility that the rate of decline in E^{vc} and E^{vc}_K is determined by an incomplete seal between the electrode and the membrane; if this was the case E^{vc} and E^{vc}_K determined initially in a new penetration should be higher than those determined just before withdrawal of the electrode from the previously penetrated cell. In some experiments the electrode was withdrawn from the ouabain poisoned tissue and placed within 5 μm from the ventricular cell membrane. In this case an apparent K^+-concentration of up to 5 mM could be observed. The concentration decreased to 2 mM when the electrode was withdrawn 300 μM into the bulk solution.

The initial rate of fall in K_c^+ in the ouabain-treated epithelium is due to the net passive leak of K^+ from the cell across a membrane or membranes with total permeability $\bar{P}_K$. This permeability may be calculated from the constant field equation[3] which expresses the flux as the product of a permeability $\bar{P}_K$ and a driving force f^{vc}. In the poisoned tissue, the net efflux J_K becomes:

[3] The Goldman equation can be obtained from integration of the Nernst-Planck flux equation, under the assumption of a constant field in the membrane. The Goldman equation can be used in a quasi-steady state, if we assume that the flux is constant through the membrane.

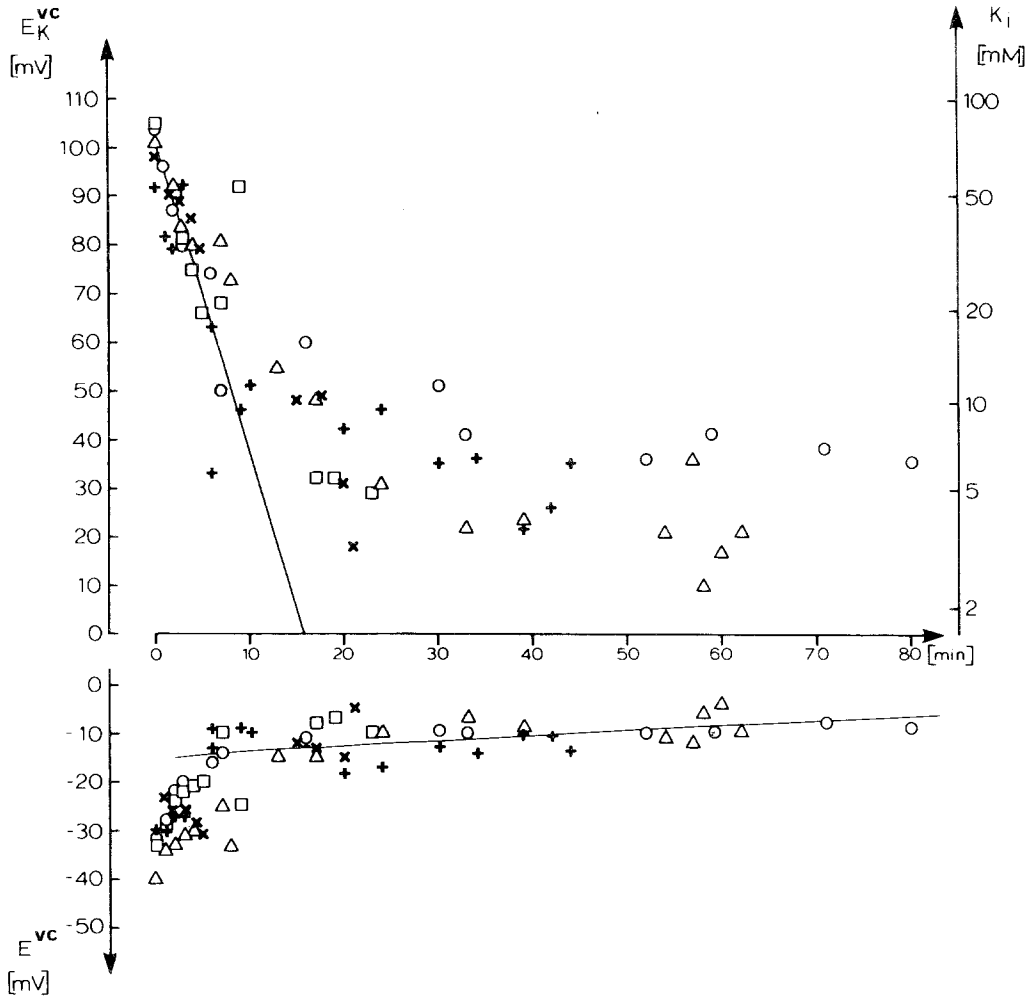

Fig. 9. The long-term effects of ouabain (10^{-4} M) on the intracellular electrical potential E^{vc}, the intracellular chemical potential E_K, and on the apparent-intracellular K^+ concentration. Numbers compiled from five animals, each shown with an individual symbol. E_K fell initially with a half-time of about 9 min (or a half-time for the apparent concentration of about 3 min). Apart from the initial abrupt decrease in E^{vc}, from about −45 to −35 mV, which is not shown here, E^{vc} decreased within 10 min to about −12 mV and remained virtually constant

$$J_K = -dK_c^+/dt \cdot h = \frac{-\bar{P}_K \cdot E^{vc} \cdot F}{RT} \cdot$$

$$\frac{K_v^+ - K_c^+ \cdot \exp(E^{vc}F/RT)}{1-\exp(E^{vc}F/RT)} = -\bar{P}_K \cdot f^{vc}. \quad (1)$$

With a cell height (h) of 10 μm, an initial dK_c/dt of 0.46×10^{-6} M·s^{-1}·cm^{-3}, $E^{vc} = -33.9$ mV, $K_v^+ = 2.0$ mM and $K_c^+ = 90$ mM, the equation yields a $\bar{P}_K$ value of 1.12×10^{-5} cm·s^{-1} (expressed per cm^2 of epithelial surface area). Using this estimate of $\bar{P}_K$, and values of E^{vc} and K_c^+ for the unpoisoned tissue, the net passive potassium efflux, which in the steady-state approximately equals the active influx, may be calculated from an equation similar to Eq. (1). This amounts to 0.41×10^{-9} mol cm^{-2} sec^{-1}.

The rate at which the K^+-activity decreased inside the ouabain-poisoned cells was a function of the K^+ activity of the ventricular solution: When K_v^+ was 2 mM, the initial rate of decline (dK_c/dt) was 0.46×10^{-6} mol cm$^-$ sec^{-1} (corresponding to a change in E_K of 0.11 ± 0.038 mV sec^{-1} SD, $n=5$), but when K_v^+ was 10 mM K^+ the rate was 0.12×10^{-6} mol cm$^-$ sec (corresponding to a change in E_K of 0.023 ± 0.013 mV sec^{-1} SD, $n=4$, which is significantly different from the former, $P<0.005$). Also if K_v^+ activity was changed from 2 to 10 mM 2 to 3 min after ouabain had been added, the rate of K^+ loss from inside the cell decreased about fourfold (Fig. 10).

2. The effects of increasing the K^+-concentration in the ventricular solution. When K_v^+ was changed to either 5, 10, 20, or 50 mM, E^{vc} depolarized abruptly by about 3, 7, 9.5, and 11 mV, respectively (Fig. 11). The depolarization was complete within a time which

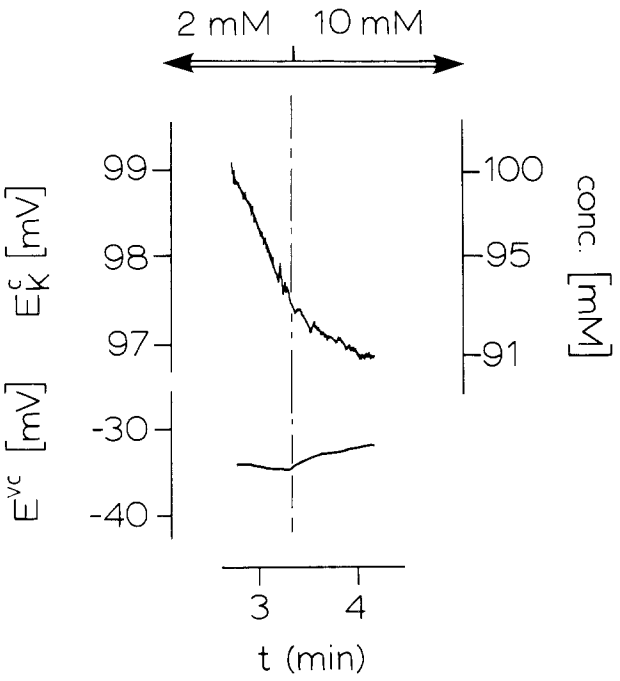

Fig. 10. The effect of changing the ventricular K^+-concentration on the rate of the ouabain induced decrease in apparent intracellular K^+concentration. At $t=0$ ouabain was administered to the ventricular solution and E_K began to decrease at a rate of about 2 mV min^{-1}. At $t=3$ min 20 sec the ventricular concentration was raised to 10 mM, which caused the K^+ to leave the cell at a slower rate, equivalent to a decrease in E_K of about 0.5 mV min^{-1}

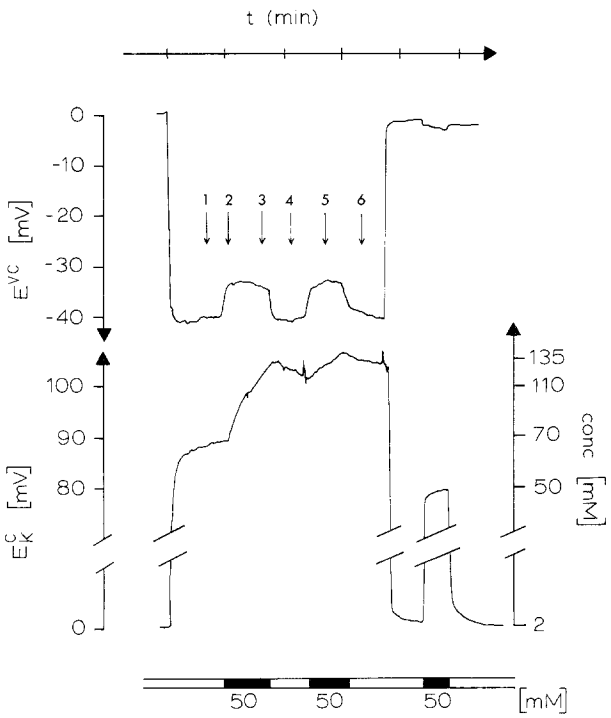

Fig. 11. The effect of increased ventricular K^+ concentration K_v^+. When the electrode had recorded a stable potential for about 1 min K_v^+ was increased from 2 to 50 mM, the intracellular electrical potential E^{vc} depolarized, and K_c^+ increased. When K_v^+ was returned to 2 mM K_c^+ decreased but at a slower rate. At different times (indicated by arrows marked 1 to 6) the ratio between the driving force f^{vc} across the ventricular membrane and the rate of increase in the K^+ could be calculated (see Eq. (1) p. 117). The epithelium was exposed twice to a K_v^+ of 50 mM, then the electrode was withdrawn to the ventricular solution and K_v^+ was increased for a third time to 50 mM in order to (i) check the electrode, (ii) measure the rate of change of the ventricular solution, and (iii) measure the transmural electrical potential difference during the exposure to asymmetrical solutions

was comparable to the time it took to change the ventricular solution (< 3 sec). The abrupt depolarization of E^{vc} as a function of the change in ventricular chemical potential of K^+ ($\Delta E^{vc}/E_K^{vc}$) is shown in Fig. 12. The measured slope was 0.13 which compared well with the value of 0.11 derived from the nonsteady-state model derived by Lew et al. (1979) under the assumptions that $\bar{P}_K$ is entirely located on the ventricular membrane and $\bar{P}_K$ is about ten times larger than the $\bar{P}_{Na}$ and $\bar{P}_{Cl}$. When K_v^+ was changed to 112 mM, E^{vc} depolarized first abruptly and linearly within 3 sec to -26 mV, then exponentially within 40 sec to about -9 mV. The transition from the linear to the exponential mode of decrease was difficult to determine more accurately than to within 5 mV.

If recordings were selected in which both the chemical and electrical potential did not change more than 0.5 mV min^{-1} in the period before and after the change in K_v^+, it could be seen that E^{vc} changed to more negative values during the application of the high K_v^+ and remained hyperpolarized after the K_v^+ was returned to 2 mM. In such cases K_c^+ was also higher after termination of the period of increased K_v^+; in fact E_K^{vc} increased by as many mV as E^{vc} had hyperpolarized (Fig. 15).

During the period of elevated K_v^+, K_c^+ increased with time. When K_v^+ was returned to 2 mM, K_c^+ tended to return to the level it had before K_v^+ was increased, but this happened at a slow rate compared to the increase (Fig. 11). To treat this quantitatively, let us first tentatively assume that the serosal membrane is impermeable to K^+. Then the net flux of K^+ into or out of the cell (J_K) can be expressed as the product of a driving force f^{vc} across the ventricular membrane and the permeability P_K^{vc} (*see* Eq. (1), p. 117). For each cell J_K was a linear function of f^{vc}. For example in Fig. 11 when K_v^+ was changed from 2 to 50 mM and back to 2 mM then J_K as a function of the driving force f^{vc} was given by the points numbered from 1 to 6 (*see* Figs. 11 and 13). The points fell on a straight line with a slope 2.5×10^{-5} cm sec^{-1} per cm^2 of cells ($r=0.94$), Fig. 13. On average the slope was 2.0 ± 0.6 10^{-5} cm sec^{-1} (11 cells, the average regression coefficient $r=0.85$). For f^{vc} equal to zero (no net driving force across

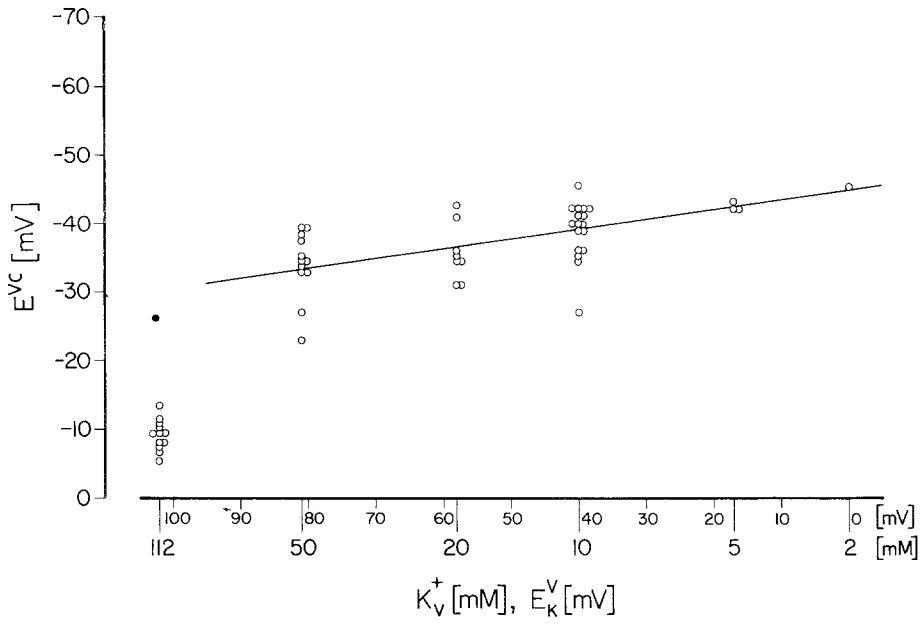

Fig. 12. The abrupt depolarization in E^{vc} as a function of a change in K_v^+ from 2 mM to either 5, 10, 20, 50, or 112 mM. The point at 2 mM is the average from 74 cells in 13 animals and had a value of -45.2 mV$\pm$2.1. All measurements are normalized relative to this point. The line is the line of least squares and is based on the experimental point from the interval 2 to 50 mM only; it had a slope of 1.3. When K_v^+ was increased to 112 mM the cell would depolarize abruptly to an average of roughly -27 mV, indicated in black, then exponentially to about -9 mV

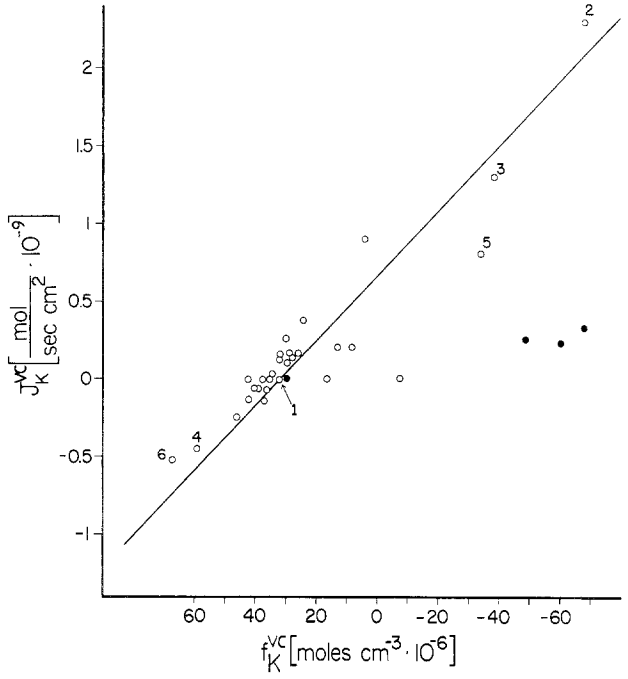

Fig. 13. The flux of K^+ into the cells J_K^{vc} (per cm^2 of cells) as a function of the driving force f^{vc} across the ventricular membrane as defined in Eq. (1) (p. 117). The serosal membrane was assumed to be impermeable to K^+. The points marked 1 to 6 were calculated from Fig. 11. The slope of the regression line was 2.0×10^{-5} cm sec^{-1} ($r = 0.85$ on average). The black points represent measurements from a cell where K_c^+ was relatively unaffected by changes in K_v^+. When $f^{vc} = 0$, J_K equals 0.54×10^{-9} mol cm^{-2} sec^{-1}

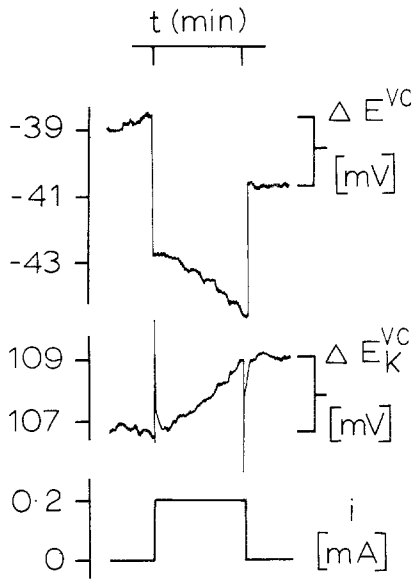

Fig. 14. The effect on the intracellular electrical potential (E^{vc}) and intracellular K^+ concentration by transmurally induced current flow. The current density i_T was 1.04 mA cm^{-2}, and was applied for 1 min. It is seen that the induced hyperpolarization (in mV) equals the increase in the chemical potential E_K, about 2 mV

the ventricular membrane) J_K was 0.54×10^{-9} moles cm^{-2} sec^{-1}. If the serosal membrane is impermeable to K^+ this is the rate of active K^+ pumping into the cell.

3. The effects of transepithelial currents. When a current of density i_T [mA cm^{-2}] was passed across the epithelium, K_c^+ increased when the current was passed from the ventricular to the serosal side (Fig. 14) and decreased when the current was reversed indicating that $g_K^{vc} > g_K^{cs}$, *see also* Zeuthen (1981). The current density was determined as described in the Methods and page 115. Within the limits of K_c^+-changes explored (roughly ± 30 mM) K_c^+ (and the chemical po-

Table 5. Parameters of K^+ transport

Cell No.	E^{vc} (mV)	E_K (mV)	Conc. (mM)	i_T (mA cm^{-2})	J_K (mol cm^{-2} sec$^{-1}\times 10^{-11}$)	i_K/i_T (%)	P^{vc} (cm sec$^{-1}\times 10^{-5}$)	g_K (Ω^{-1} cm$^{-2}\times 10^{-3}$)
1	−53	102	113	3.12	36.7 ±9.3 (6)	1.12 ±0.26 (6)	6.1 ±2.5 (6)	5.7 ±1.2 (6)
2	−48	93	80	0.52	43.3 ±14.7 (6)	10.9 ±4.9 (6)	12.6 ±2.7 (5)	18.1 ±5.4 (5)
3	−53	108	144	0.52	50.5 (2)	9.1 (2)	4.5 (2)	7.3 (2)
4	−42	105	127	0.52	61.5 ±30.8 (6)	9.1 ±4.7 (6)	5.8 ±1.4 (4)	14.3 ±5.8 (4)
5	−45	97	92	1.04	12.0 (1)	1.12 (1)	2.0 (1)	1.6 (1)
6	−46	86	61	1.04	7.9 (1)	0.74 (1)	4.4 (1)	2.5 (1)
7	−45	87	63	1.04	4.2 (1)	0.39 (1)	0.6 (1)	1.4 (1)
8	−50	88	66	1.04	10.2 ±2.8 (3)	0.94 ±0.18 (3)	4.7 ±2.4 (3)	4.3 ±0.37 (3)
9	−45	86	61	1.04	10.6 (1)	0.98 (1)	8.8 (1)	5.1 (1)
$\bar{x}$	−47 ±3.9 (9)	95 ±8.7 (9)	90			3.8 ±4.5 (9)	5.5 ±3.6 (9)	6.7 ±5.7 (9)

i_T is the current density at the flat part of the epithelium. J_K is the corresponding K flux into the cell and is estimated from dK_c^+/dt. i_K/i_T is the ratio of current carried into the cell to the total current. P_K^{vc} is the permeability of the ventricular membrane synonymous to a partial conductance g_K. All numbers are ±SD. The numbers in the parantheses are the number of experiments in each cell. The lower line is the average of all cells.

tential E_K^{vc}) changed roughly linearly with the time of current-application. Table 5 shows the results obtained in nine cells (six animals). The rate of change in K_c^+ times Faraday's constant $[i_K=(dK_c^+/dt)\cdot F]$ represents the current carried by K^+ ions across the ventricular membrane minus the current carried by K^+ ions across the serosal membrane. On average this current was 4% of the total current across the epithelium. From Eq. (1) (p. 117), the assumption that the serosal membrane is impermeable to K^+, and the nonsteady-state model of Lew et al. (1979), P_K^{vc} and g_K (the partial conductance of K^+ across the ventricular membrane) could be calculated during the passage of the current into the tissue. P_K^{vc} was on average 5.5×10^{-5} cm sec^{-1} and g_K was 6.7×10^{-3} Ω^{-1} cm^{-2} (equivalent to an impedance of 150 Ωcm^2). When current was passed from the blood side into the ventricle (negative currents) P_K and g_K were about 20% lower than for positive currents[4].

[4] This difference is probably due to polarization effects (Bindslev, Tormey & Wright, 1974).

4. The effects of increased K_c^+. During the exposure of the epithelium to either elevated K_v^+ (p. 117) or to positive currents (p. 119). K_c^+ increased and E^{vc} changed towards more negative values (in the case of increased K_v^+ the change in E^{vc} was superimposed upon the initial abrupt depolarization). If we look at only those cases where the induced changes in E_K^{vc} and E^{vc} were less than 5 mV (typically 3 mV) (*see* Figs. 11 and 14), E^{vc} and K_c^+ (E_K^{vc}) appeared to remain constant (change <0.5 mV min^{-1}) after the termination of the external changes. The hyperpolarization in E^{vc} in mV was equal to the increase in E_K^{vc}, Fig. 15, which indicates that K^+ is the ion which has the highest electrodiffusive permeability as compared to other ions.

Discussion

First, we review the steady-state distribution of K^+ within the tissue, and then discuss the evidence for the location of the potassium pump in the epithelium.

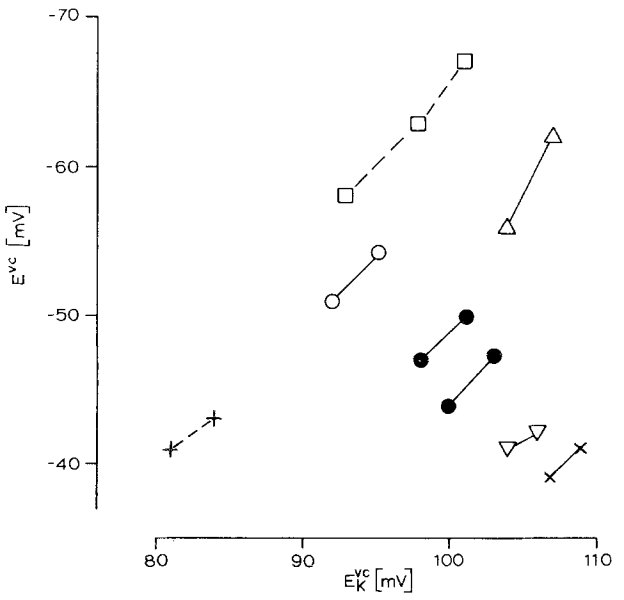

Fig. 15. The apparent steady-state values of E^{vc} and E_K before and after K_c^+ had been increased by either elevating K_c^+ (broken lines) or passing a positive current (full lines) across the epithelium. An increase in E_K was accompanied by a numerically equal change in E^{vc}. Different symbols indicate different animals

Next we describe the properties of the pump in the brush border membrane. This is followed by a discussion of the mechanisms of passive K^+ transport across the brush border and basolateral membranes. Finally, we place these results into the perspective of the intact epithelium and conclude with a statement of the relevance of our model and to transport of ions across other epithelia.

Distribution of K^+ within the Plexus

Chemical experiments (p. 111) indicate that the intracellular potassium concentration in the plexus is about 154 mM. Using ion-specific microelectrodes (p. 115) on the other hand gives an intracellular concentration of 90 mM for the epithelial cells. The discrepancy between the two sets of experiments is in part due to uncertainties about intracellular spaces and compartmentalization of ions within the tissue. Experiments with ^{42}K suggest that, (i) ~40% of the total K in the tissue is confined to the epithelium, and (ii) ~40–55% of the potassium in the plexus is readily exchangeable with isotope in the incubation media (*see* Nellans & Schultz, 1976; Robinson & Macknight, 1976).

Both the tracer and the electrophysiological experiments indicate that there are two pools of K within the epithelium, i.e., efflux experiments (Figs. 4 and 9) show that the intracellular K_c^+ washes out in two phases. One phase is determined by the cellular compartment which was accessible to the electrode. However, there are no definite clues about the identity of the other compartment: One possibility is the unstirred layer adjacent to the ventricular membrane (Fig. 8), while another is the subcellular organelles or stromal compartment. It should be noted that up to 20–50% of the cellular volume is occupied by subcellular organelles such as the nucleus and mitochondria. In other cells there is evidence that the K^+ concentration in these organelles is at least as high as that in cytoplasm (*see* Moore & Morrill, 1976; Gupta, Hall & Naftalin, 1978; Rick et al., 1978*a*, *b*). The absence of information about the K^+ content and permeability of these organelles in the plexus makes it difficult to draw more definite conclusions about their role in the K^+ compartmentalization.

Chemical analysis also indicates that the intracellular Na^+ concentration is 17 mM in the plexus (Wright, 1978*b*), but this value must be inaccurate in view of similar uncertainties about the compartmentalization of this ion. Preliminary experiments using Na^+ specific electrodes yield a concentration of 8.5 mM.

As in single cells and other epithelia the high intracellular K^+ concentration in the plexus is accounted for by active transport. First, the potassium electrochemical potential difference between the cytoplasm and the external solutions was −95 mV, whereas the electrical potential difference was only −47 mV (*see* Table 5). Although in general this difference between E_K^{vc} and E^{vc} could be due to an underestimate of the membrane potential (Zeuthen, 1980), this does not appear to be true in the present case since E^{vc} determined with the double-barrelled electrodes is no less than that determined in the same tissues with high impedance single-barrelled electrodes (p. 114). In addition, K^+ is not at equilibrium between the extracellular and intracellular compartments in the gallbladder and small intestine (*see* Zeuthen, 1978). Second, ouabain, a potent inhibitor of Na/K pumps, abolished the K concentration gradients (Figs. 8 and 9; and Fig. 8, Wright, 1978*b*) and the value for E_K^{vc} approached E^{vc} (Fig. 9). Finally, the intracellular accumulation of potassium was abolished by incubation of the plexus at 0 °C in K-free saline (Wright, 1978*b*, Fig. 8; Zeuthen & Wright, 1978, Fig. 2).

On the basis of these experiments it is concluded that K^+ is actively accumulated within the choroidal epithelium against its electrochemical potential gradient by a ouabain-sensitive pump.

Location of the Pump

Three sets of experiments place the pump in the brush border membrane. (i) ^{42}K uptake: The rate of oua-

bain-sensitive ^{42}K uptake into the choroid plexus across the ventricular surface was 15 times greater than that across the serosal surface (p. 107). (ii) K-sensitive microelectrodes: Ouabain added abruptly (within 1–3 sec) to the ventricular solution rapidly depolarized electrical potential E^{vc} (Fig. 8). This was followed by a decrease in K_c^+. Only if the pump was located at the ventricular membrane is such a fast effect of ouabain possible. In another type of experiment plexuses were depleted of K^+ by storage in cold K^+-free solutions (Zeuthen & Wright, 1978, Fig. 2). The reintroduction of K^+ to the ventricular solution within 1–3 sec immediately increased K_c^+, at a rate comparable to the normal pump rate (about 0.36×10^{-9} mol cm^{-2} sec^{-1}). (iii) ^{3}H-ouabain binding: Quinton, Wright and Tormey (1973) showed by autoradiography that ouabain sites were restricted to the brush border membrane of the epithelium. The binding of ouabain to the basolateral membranes of the epithelium and the supporting connective tissue was not above background levels.

Mechanism of K Pumping Across the Brush Border Membrane

Relationship to Na^+. There are two arguments to link active K^+ transport across the brush border membrane to active sodium secretion. The first is that ouabain in the CSF inhibits: (i) active K^+ accumulation (p. 109); (ii) active sodium secretion (Wright, 1972*b*); and Na/K-ATPase activity (Vates, Bonting & Oppelt, 1964; Wright, 1978*b*, Fig. 7). The second argument is that active K accumulation requires the presence of Na: (i) K transport is inhibited 70% when the tissue is preincubated in saline where the sodium is reduced to 5 mM by replacing NaCl with choline chloride (Table 2); and (ii) the intracellular Na and K concentrations depend on the simultaneous presence of both Na and K (Wright, 1978*b*, Fig. 8).

Kinetics of K pumping. K^+ transport is a sigmoid function of K concentration and the kinetic analysis suggest the pump has two K binding sites (Fig. 3). The K_m and V_{max} for this two-site model are 0.3 mM and 8 μmol cm^{-2} hr^{-1}. These kinetics are similar to those observed for K uptake into red blood cells and nerve and for K effects on the Na/K-ATPase (*see* Schwartz et al., 1975).

Ouabain binding. ^{3}(H)-ouabain binding to the brush border membrane of the plexus can be fitted to a curve with a K_m of 8×10^{-7} M and a B_{max} of 4.5×10^{-11} moles per plexus (Fig. 5). This corresponds to 1×10^{-11} mol/mg wet wt, which was comparable to that obtained for rabbit renal tubules (Shaver & Stirling, 1978), and to 10×10^6 sites per cell assuming that folding of the tissue increases the surface area by a factor of 10 (*see* below). By way of contrast, there are only about 250 sites on each human red blood cell (e.g., Erdmann & Hasse, 1975).

Ouabain inhibition of K transport. Kinetics of ouabain inhibition of K uptake by the plexus (Wright, 1978*b*, Fig. 11) give a K_m of 8×10^{-7} M, and complete inhibition of K pumping is produced by an ouabain concentration of 7×10^{-6} M.

Turnover of the K pump. Estimates of pump turnover times can be obtained by comparing the kinetics of ouabain inhibition of the K pump and the kinetics of ouabain binding to the plexus. Plotting the inhibition of K transport against the amount of ouabain bound gives a slope of 10 sec^{-1}, i.e., if 1 ouabain molecule is bound to each pump site each pump turns over 10 sec^{-1} at 22 °C. A comparable turnover time can be estimated from the V_{max} for K uptake (1.7×10^{-9} moles cm^{-2} sec^{-1}) and the B_{max} for ouabain binding (3.5×10^{-10} mol cm^{-2}), i.e., 5 sec^{-1}. These estimates are similar to those obtained for rabbit vagus nerve at 22 °C (22 sec^{-1}) by Landowne and Ritchie (1970) and a variety of other cells and tissues (50–200 sec^{-1}) at 37 °C (*see* Harms & Wright, 1980). The turnover rates may vary by a factor of four depending on whether 1 or 2 ouabain molecules are bound per transport site and whether 1 or 2 K ions are transported per cycle of the pump.

The electrogeneicity of the Na/K-pump. The fact that ouabain caused an abrupt depolarization of 10.2 mV strongly suggest that the Na/K-pump is electrogenic. The depolarization was complete in 5 to 10 sec which indicates a binding as fast as that observed in axons (Baker & Manil, 1968; Baker & Willis, 1972). The magnitude of the depolarization is indicative of a coupling-ratio $r = 1.5$ between the active Na-efflux and K^+-influx mediated by the pump; it can be shown (Zeuthen, 1981) that the maximal obtainable depolarization induced by ouabain is equal to $RT/F \cdot \ln r$ which in the case of $r = 1.5$ becomes 10.4 mV. In agreement with this Frömter and Gessner (1975) observed in the rat kidney tubule cells an abrupt depolarization of 10.6 mV ± 2.0 ($n = 14$) complete in about 5 sec when ouabain was added to the peritubular perfusion solution, values surprisingly close to ours.

On the basis of these results and those from the kinetic of potassium transport (see p. 108), we conclude that three sodium ions are pumped out of the cell for every two potassium ions pumped in.

Permeability of Cell Membranes

Unidirectional fluxes. In the steady-state the unidirectional fluxes across each cell membrane of the choroidal epithelium are related to the unidirectional fluxes across the following expressions (*see* Ussing & Zerahn, 1951; Wright, 1974):

$$J^{net} = J^{vs} - J^{sv} = J^{vc} - J^{cv} = J^{cs} - J^{sc}$$

and

$$J^{vs} = \frac{J^{vc} \times J^{cs}}{J^{cv} + J^{cs}}$$

and

$$J^{sv} = \frac{J^{sc} \times J^{cv}}{J^{cv} + J^{cs}}$$

where J^{net} is the net flux across the epithelium, J^{vs} and J^{sv} the unidirectional fluxes across the epithelium, J^{vs} and J^{cv} the unidirectional fluxes across the basolateral membranes. Superscripts *v, s,* and *c* refer to the ventricular serosal and cellular compartments, e.g., J^{vc} is the unidirectional flux from the ventricular to the cellular compartment.

To obtain a complete description it is necessary to take into account the magnitude of the unidirectional fluxes through the shunt pathway, i.e., the fluxes that bypass the cells and then cross the epithelium through the tight junctions and the lateral intercellular spaces. As a working hypothesis we assume that 95% of the passive unidirectional fluxes across the epithelium i.e., $0.95 \times J^{sv} = 0.95 \times 0.025 \times 10^{-9}$ mol cm^{-2} sec^{-1} $= 0.021 \times 10^{-9}$ mol cm^{-2} sec^{-1}, proceeds via the shunt pathway (for justification *see* below).

Taking the experimental values obtained for the potassium fluxes, i.e., $J^{vs} = 0.033$, $J^{sv} = 0.025$, J^{vc} 0.79 and $J^{shunt} = 0.021$ nmol cm^{-2} sec^{-1} (Wright, 1972, 1978*b*; and p. 107), the remaining unidirectional fluxes are estimated from the above equations to be $J^{cv} = 0.79$, $J^{cs} = 0.01$, $J^{sc} = 0.002$ and $J^{net(vs)} = 0.008$ nmol cm^{-2} sec^{-1}.

A summary of the unidirectional fluxes is given in Fig. 16 and Table 6. The most interesting conclusion is that most of the K transported into the epithelium by the pump in the brush border membrane leaks back into the ventricular fluid across the same membrane. This is consistent with the ^{42}K washout studies which predicts a value of 0.47 nmol cm^{-2} sec^{-1} for this efflux. A clue about the relative permeability of the brush border and basolateral membranes may be obtained from the ratio of the unidirectional fluxes across each face of the epithelium, i.e., $P_K^{cv}/P_K^{cs} = J_{cv}/J_{cs} = 70$ (as the driving forces, concentration gradients, and electrical potentials, are virtually identical across each membrane). This leads to the conclusion that the brush border membrane is much more permeable to potassium than the basolateral membrane (*see* below). The precise ratio of the P_K's depends on the actual permeability of the shunt path, since the calculated unidirectional fluxes across the serosal membrane are quite sensitive to variations in the fluxes through the junctions. Nevertheless, the ratio of permeabilities are compatible with that deduced from the electrophysiological studies (*see* below).

Electrophysiology. The electrophysiological experiments show that the epithelial cell membrane (ventricular plus serosal) has an electrodiffusive permeability for K^+ which is much larger than that for other ions: When the intracellular K^+-activity was increased or decreased either by passing a current transepithelial or by changing K_v^+ (*see also* Zeuthen, 1981), the intracellular electrical potential E^{vc} hyperpolarized or depolarized by as many mV as the chemical potential (E_K^{vc}) had changed (Figs. 14 and 15). Experiments with transmural currents (p. 119) and measurements of the voltage divider ratio α (p. 115) show that this K^+-permeability is situated predominantly at the ventricular membrane. The conductivity of the ventricular membrane was between 9–6 times more conductive than the serosal membrane. From the relation between α, partial conductances, and permeabilities which can be derived from expressions similar to Eq. (1) (p. 117), it can be shown that P_K^{vc} must be 15–21 times P_K^{cs} if it is assumed that the electrodiffusive components of the Na^+ and Cl^- permeabilities are situated at the serosal membrane and assumed to be of the order of 1/10 of the K^+ permeability. In the case that they were located at the ventricular membrane, which is unlikely in view of the direction of the transepithelial NaCl transport, P_K^{cv} would be only 4–6 times that of P_K^{cs}.

The other types of electrophysiological experiment are also consistent with the finding of a passive electrodiffusive leak for K^+ situated at the ventricular membrane.

a) When K_v^+ was increased from 2 to between 5 and 50 mM, E^{vc} depolarized abruptly by 0.13 mV for each mV increase in the chemical potential E_K^{vc} (Fig. 12). The nonsteady-state model derived by Lew et al. (1979) predicts a 0.11 mV depolarization for each mV increase in the chemical potential but only under the assumptions (i) that the K permeability of the cell was located at the ventricular membrane, (ii) that $P_K > P_{Na}$, P_{Cl}, and (iii) that current flowing across the serosal membrane returns to the ventricular fluid via a leaky tight junction (*see* below).

b) When the rate of increase in K_c^+ is plotted *vs.*

the driving force for K^+ across the ventricular membrane, f_K^{vc} (Fig. 13), a straight line is obtained. This is what would be expected if $P_K^{vc} \gg P_K^{cs}$, and the slope of the line would then be an estimate of the permeability of the ventricular membrane and the intercept of $f_K^{vc}=0$ would indicate the magnitude of the rate of active K^+ transport. If the serosal membrane had a significant permeability to K^+ (P_K^{cs}), the slope of the J_K *vs.* f_{vc} plot would have been a line curving downwards. If, for example, $P_K^{cs}=P_K^{vc}$ the slope at negative values of f^{vc} would be about four times larger than at positive values. No significant difference was found between the slopes for negative and positive values of f^{vc} and this suggests that P_K^{cs} is insignificant compared to P_K^{vc}.

c) No unstirred layer is observed when the electrode is advanced onto the ventricular membrane of the unpoisoned tissue (Fig. 8). If the K which was pumped into the cell from the ventricular solution continued into the serosal compartment, then the K_v^+ adjacent to the membrane should be lower than in the bulk CSF.

d) From the magnitude of the unstirred layer (120 μM, Wright, 1974), and the K concentration observed at the ventricular membrane of a tissue poisoned with ouabain (*see* Fig. 8), it can be calculated from Fick's law, assuming a flat geometry of the tissue, that the flux of K^+ away from the ventricular unstirred layer into the bulk CSF is of the order of 4×10^{-9} mol cm^{-2} sec^{-1}. Thus the rate of decrease of the intracellular K^+ concentration 0.46×10^{-6} M sec^{-1} cm^{-3} can amply be explained by an efflux of K^+ across the ventricular membrane alone. It should be emphasized, however, that any comparison between these two estimates of the efflux is complicated by the fact that the high K^+ in the unstirred layer may come partly from cells at the bottom of folds and villi (those cells which are exposed to the ouabain later than those on the rims) as well as cells from the flat part of the epithelium.

e) The rate at which K^+ decreased in the ouabain-poisoned tissue was influenced by the K^+ concentration in the ventricular solution. With $K_v^+=10$ mM we can calculate the expected efflux across the ventricular membrane from the permeability and an intracellular electrical potential. Increasing K_v^+ from 2 to 10 mM should decrease dK_c^+/dt from 0.46×10^{-6} mol cm^{-2} sec^{-1} to 0.38×10^{-6} mol cm^{-2} sec^{-1}. However, we observed a decrease to as low as 0.12×10^{-6} mol cm^{-2} sec^{-1}. Thus not only does the change from 2 to 10 mM K in the ventricular solution account for the change in the rate of efflux, but it appears that the potassium permeability of the ventricular membrane itself decreases by a factor of about 4 (to 0.3×10^{-5} cm sec^{-1}), when exposed to both ouabain and high external potassium. Admittedly there could be other explanations for this apparent decrease in permeability, but further experiments are needed on this point.

All the estimates of P_K^{vc}, P_K^{cs} and P_K^{vc} are compared in Table 6.

Model of the Epithelium

Although K^+ is transported across the plexus from CSF to blood, the major function of the epithelium is to secrete fluid. Na^+ is actively transported from blood to CSF at a rate of 0.28 nmol cm^{-2} sec^{-1}, and this is accompanied by the net movement of Cl^-, HCO_3^- and water into the CSF. These transport processes occur in the absence of any significant electrical potential difference across the plexus (*see* Wright, 1978*a* for a review). The absence of a potential is due to the existence of a high conductance pathway between the epithelial cells, i.e., the so-called tight junctions of the choroid plexus are leaky to small ions.

Evaluation of the paracellular shunts. The transepithelial impedance was estimated to be 26 Ω cm^2 when current was passed across the tissue. The PD drop across the epithelium was determined using two microelectrodes; one was placed in the connective tissue just behind the epithelium (within 10 μm of the basement membrane), and the other was in the ventricular solution less than 50 μm in front of the brush border membrane. The actual current density was obtained by scanning the electrical field in the ventricular solution. The difference between this value as the impedance of the epithelium and that reported for the entire plexus (200 Ω cm^2), (Wright 1972*a*; *see also* p. 115) is due to folding of the tissue and the resistance of the connective tissue layer.

The quantitative significance of the shunt to the low impedance can be assessed as follows:

a) Effect of transmural currents of K_c^+. From the rate of increase of K_c^+ during passage of transepithelial current the K permeability of the ventricular membrane (P_k^{vc}) was determined as 5.5×10^{-5} cm sec^{-1}. This is equivalent to a partial conductances g_K^{vc} of 6.7×10^{-3} Ω cm^{-2}, under the reasonable assumption (*see* above) that $P_K^{vc} > P_K^{cs}$. Making the further reasonable assumption that $P_K > P_{Na}, P_{Cl}$ (*see* p. 120), it seems that the current carried across the ventricular membrane is largely carried by K^+. As we know this current is 4% of the total transepithelial current (Table 5), we can conclude that the transepithelial shunt carries 96% of the transepithelial current. This is close to the value obtained by Frömter (1972) for the gall-

bladder. The agreement between the two estimates is surprising in view of the difference in the two methods and the inaccuracies involved (*see* Zeuthen, 1976, for a critical evaluation of cable analyses of epithelia).

b) Variation in membrane potential with changes in CSF K^+. Changes in CSF K^+ concentration depolarize the membrane potential across the brush border membrane abruptly by 0.13 mV per mV change in E_K^{vc} (*see* Fig. 12). On the other hand, current-induced changes in K_c^+ produce the variations in membrane voltage expected for a K-selective membrane, i.e., $dE^{vc}/dE_K^{vc} \sim 1$ (Fig. 15). This apparent discrepancy is accounted for by a nonsteady-state model (Zeuthen, 1981) which predicts that dE^{vc}/dE_K^{vc} is 0.11 mV/mV, which is close to the observed value of 0.13 mV/mV. Implicit in the model is the presence of a large extracellular shunt to carry current around the cell.

c) Effect of membrane potentials on the passive K uptake. Radioactive tracer experiments (Fig. 2) show that the passive uptake of potassium across the ventricular surface of the plexus is largely independent of the membrane potential across the ventricular membrane, i.e., the passive permeability is independent of variations in membrane potential of -45 to 0 mV. The permeability coefficient, $P_K = 3 \times 10^{-5}$ cm sec^{-1}, is an equivalent to a passive uptake of 0.06 nmol cm^{-2} sec^{-1} at a CSF concentration of 2 mM. This value is twice the passive unidirectional flux of K across the plexus (0.03 nmol cm^{-2} sec^{-1}). The most reasonable explanation is that a flux across the tight junctions accounts for a major fraction of the passive uptake of K across the ventricular surface of the plexus. Variations in CSF potassium concentration produce no change in voltage across the epithelium as P_K/P_{Na} for the whole epithelium is close to 1 (Wright, 1972*b*).

Other circumstantial evidence for ion permeation across the plexus through the tight junctions includes: (i) the high tissue conductance and low transmural electrical potentials; (ii) high unidirectional fluxes relative to the net fluxes; (iii) morphological studies with La^{+++} as a tracer; (iv) low discrimination between ions; and (v) the kinetics of active anion (iodide) transport (*see* Wright, 1978*a*).

Comparison of fluxes measured by tracers and electrodes. All tracer fluxes have been expressed in terms of the serosal area of the plexus, i.e., per cm^2 of the window area of the chamber. All electrode data, on the other hand, have been expressed per cm^2 of epithelial cells, assuming that the area of each cell is 1×10^{-6} cm^2. To compare the two sets of data it is necessary to know the factor by which folding increases the area of the epithelium over the serosal area. This can be obtained from the total number of cells in the plexus. There are two estimates: The first is based on the wet weight of the tissue in the chamber and the extracellular space, assuming that most of the intracellular volume is due to epithelial cells. The wet weight of the plexus is about 2.2 mg and the extracellular space is 53% of the total tissue water (p. 111), i.e., the weight of cells is ~ 1.2 mg. Since the weight of one cell is the cell volume (10 μm^3) times the density (~ 1.2 g/ml), there are about 1×10^6 cells per plexus; the second estimate is based on the cell potassium (volume $\times$ concentration $=$ 10 $\mu m^3 \times 90$ mM $= 9 \times 10^{-14}$ mol) and the total potassium content of the plexus. In a chamber the potassium content is approximately half the total for the plexus (1.3×10^{-7} mol). Assuming that 50 to 100% of the tissue potassium is in the epithelium, there are 0.7–1.4×10^6 cells per plexus. These two estimates of the number of cells together with the ventricular area of each cell (1×10^{-6} cm^2) give an epithelial area of 0.7 to 1.4 cm^2 per chamber area of 6.2 mm^2, and an amplification factor of $\times 11$ to $\times 22$. Preliminary experiments in which the electrical field in the folds of the epithelium was scanned by a microelectrode during passage of transmural current (T. Zeuthen, *unpublished*) indicates that the functional amplification factor is only threefold. Therefore, the tracer flux values in Table 6 have to be corrected by a factor of between 3 and 22. The tracer and electrode data therefore agree to within an order of magnitude.

Relation to other epithelia. Electrically, the choroidal epithelium (26 Ωcm^2) can be classified as a very low resistance epithelia within the group of cold blooded animals. It is lower than the gallbladder of frog (114 Ωcm^2, Bindslev, Tormey & Wright, 1974) and *Necturus* (207 Ωcm^2, Frömter, 1972) and the proximal tubule of *Necturus* kidney (43–155 Ωcm^2, Spring & Paganelli, 1972). It is only more resistant than the endothelium of the capillary wall of the frog mesentery 5 Ωcm^2 (Crone, 1980). Compared to the epithelia of the warm-blooded animals it is comparable to the rabbit gallbladder (28 Ωcm^2, Wright & Diamond, 1968) and has a larger impedance than the proximal tubule of the rat (5 Ωcm^2, Hegel, Frömter & Wick, 1967) and dog (6 Ωcm^2, Boulpaep & Seely, 1971).

Conclusion

A summary of the potassium fluxes across the epithelium and across each of the plasma membrane is given in Fig. 16 in conjunction with Table 6.

At a normal K concentration of 2 mM in the saline, potassium is transported into the epithelium by a

Table 6.

Method	Permeability (cm sec^{-1})	Pumping rate (mol cm^{-2} sec^{-1})	Coupling ratio
Electrophysiology[a]			
Ouabain	$P_K = 1.04 \times 10^{-5}$	$p_K^{vc} = 0.41 \times 10^{-9}$	$r = 1.5$
Ouabain +high K_v^+	$P_K^{vc} = 0.31 \times 10^{-5}$		
High K_v^+	$P_K^{vc} = 2.1 \times 10^{-5}$	$p_K^{vc} = 0.55 \times 10^{-9}$	
Transmural current	$P_K^{vc} = 5.5 \times 10^{-5}$		
Voltage divider ratio	$P_K^{vc} = 15\text{–}21 \times p_K^{cs}$		
K-depletion[c] (Zeuthen & Wright, 1978)		$p_K^{vc} = 0.36 \times 10^{-9}$	
Unstirred layer		$p_K^{vc} \sim 4.0 \times 10^{-9}$	
Tracer study[b]			
^{42}K efflux	$P_K^{cv} > 1.5 \times 10^{-5}$	p_K^{vc} 0.47×10^{-9}	
^{42}K influx		p_K^{vc} 0.79×10^{-9}	2 K ions per pump cycle
Transmural fluxes and ^{42}K influx	p_K^{cv} 2.4×10^{-5} $P_K^{cv} \sim 70 \times P_K^{cs}$ P_K^{sv} 9×10^{-6}	p_K^{vs} 0.008×10^{-9}	
Conductances and relative P's	P_K^{sv} 9×10^{-6}		

[a] All electrode expressed per cm^2 epithelium, which is 3–22 × serosal area (*see* text).
[b] All tracer expressed per cm^2 serosal area.
[c] From Zeuthen & Wright (1978).

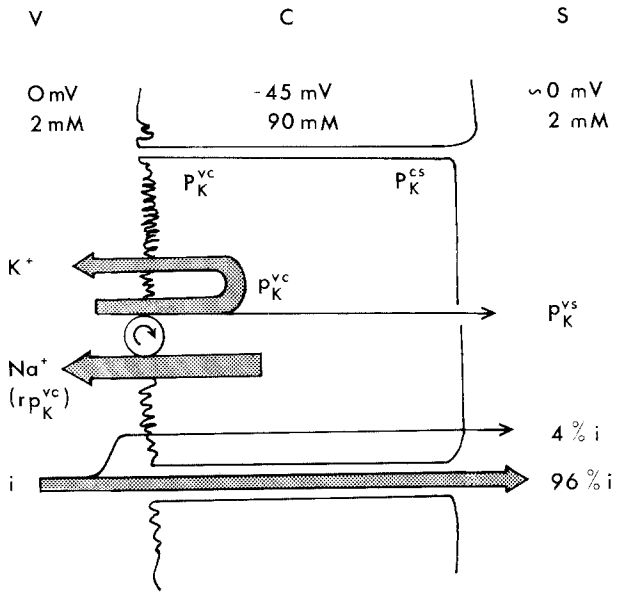

Fig. 16 and Table 6. A summary of our findings concerning K movements in the choroidal epithelium between the ventricular compartment *v*, the cellular compartment *c* and the serosal compartment *s*. The numerical values corresponding to Fig. 1 is in Table 1. Electrical potentials are indicated in mV and apparent concentrations in mM. K is pumped from *v* to *c* at a rate p_K^{vc}, only to leave the cell mainly across the mucosal membrane which has a permeability P_K^{vc}. A minor fraction p_K^{cs} leaves the cells across the serosal membrane (permeability P_K^{cs}) and is measured at the transmural flux. The active influx of K is coupled to an active efflux of Na at a rate rp_K^{vc} where r is 1.5; two K-ions are pumped for each three Na ions. The tissue is a leaky epithelia; when a current i is passed from *v* to *s* 4% of the current passes through the cell and 96% paracellulary

Na/K exchange pump in the ventricular membrane. Most of the K that is transported returns to the ventricular fluid by diffusion out across the ventricular membrane. In the steady state the electrical potential between the cell and the external solutions is −45 mV, of which the electrogenic pump contributes 10 mV. The internal potassium is held at 90 mM, which is above the equilibrium value. There is a small leak of potassium out the serosal membrane, and this accounts for the small net flux of this ion from the ventricle to the serosa. The net flux is small – owing to the low permeability of the serosal membrane to K. The high unidirectional fluxes of potassium across the epithelium, i.e., relative to the net flux, and the absence of a transmural electrical potential are due to the presence of a high conductance leak through the paracellular shunt.

Assuming that there is one ouabain molecule bound to each pump site, there are approximately 10×10^6 transport sites per cell. These sites turn over about 10 times per second, and two potassium ions are transported per cycle of the pump. The pump directly contributes to the membrane potential by 10 mV, and our analysis suggests that there are three sodium ions transported out of the cell for every two potassium ions transported in across the ventricular membrane.

We must conclude that, as far as our experiments go, the epithelium behaves as predicted by the model of Koefoed-Johnsen and Ussing (1958). In this model it is envisaged that sodium enters the epithelium across one face of the epithelium down its electrochemical potential gradient, and then it is pumped out across the other face of the cell by a sodium potassium exchange pump. It is proposed that this Na/K pump is similar to that found in single cells, and it performs the dual function of transepithelial ion transport and maintaining the intracellular sodium and potassium concentrations. Potassium which is pumped into the cell across the ventricular membrane leaves predominantly across the same membrane.

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Note Added in Proof

The relation between the depolarization caused by ouabain and the ratio of coupling of the active fluxes of Na^+ and K^+ is discussed in a letter to this journal: On the effect of amphotericin B and ouabain on the electrical potentials of the *Necturus* Gallbladder, by T. Zeuthen (*J. Membrane Biol.* **60**:167).