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# Potassium Transport Across the Frog Retinal Pigment Epithelium

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**Summary.** Previous experiments indicate that the apical membrane of the frog retinal pigment epithelium contains electrogenic Na:K pumps. In the present experiments net potassium and rubidium transport across the epithelium was measured as a function of extracellular potassium (rubidium) concentration, $[K]_o$ ($[Rb]_o$). The net rate of retina-to-choroid $^{42}K$($^{86}Rb$) transport increased monotonically as $[K]_o$ ($[Rb]_o$) increased from approximately 0.2 to 5 mM on both sides of the tissue or on the apical (neural retinal) side of the tissue. No further increase was observed when $[K]_o$ ($[Rb]_o$) was elevated to 10 mM. Net sodium transport was also stimulated by elevating $[K]_o$. The net K transport was completely inhibited by $10^{-4}$ M ouabain in the solution bathing the apical membrane. Ouabain inhibited the unidirectional K flux in the direction of net flux but had no effect on the back-flux in the choroid-to-retina direction. The magnitude of the ouabain-inhibitable $^{42}K$($^{86}Rb$) flux increased with $[K]_o$ ($[Rb]_o$). These results show that the apical membrane Na:K pumps play an important role in the net active transport of potassium (rubidium) across the epithelium. The $[K]_o$ changes that modulate potassium transport coincide with the light-induced $[K]_o$ changes that occur in the extracellular space separating the photoreceptors and the apical membrane of the pigment epithelium.

**Key words** retinal pigment epithelium · potassium transport · Na:K pumps · epithelial transport

## Introduction

In a previous study it was demonstrated that the apical membrane of the frog retinal pigment epithelium (RPE) contains a ouabain-sensitive electrogenic pump (Miller, Steinberg & Oakley, 1978). It was also shown that the voltage produced by this pump can be reduced by decreasing $[K]_o$ outside the apical membrane or by elevating potassium in the cells (Oakley, Miller & Steinberg, 1978). The evidence that this pump is located exclusively on the apical membrane has been corroborated in the frog preparation by autoradiographic experiments using tritiated ouabain (Bok, 1980). The (Na, K)-ATPase activity of these cells has been localized to the apical membrane (Ostwald & Steinberg, 1980) and this activity is consistent with the net flux of sodium across the tissue (Miller & Steinberg, 1977*a*; Riley, Winkler, Benner & Yates, 1978). It therefore seems appropriate to further compare this ouabain-inhibitable, potassium-sensitive electrogenic pump with the sodium-potassium exchange pump that has been so extensively studied in red blood cells, nerve and muscle (Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969; Garay & Garrahan, 1973; Kennedy & DeWeer, 1977*a*, *b*; Joiner & Lauf, 1978).

In the present paper we measured the active transport of potassium and sodium across the RPE as a function of $[K]_o$ and we determined the effect of ouabain on active potassium transport at several different levels of $[K]_o$. With $[K]_o = 2$ mM on both sides of the tissue, the net $^{42}K$ flux is $\simeq 2.5\%$ of the short-circuit current. [The rest of the SCC is almost entirely determined by Na, Cl and $HCO_3^-$ transport systems (Miller & Steinberg, 1977*a*; Steinberg & Miller, 1979).] The relationship between net transepithelial $^{42}K$ flux and K pump current can be inferred provided there is additional information about the "passive" movement of potassium across the apical and basal membranes. This information was previously obtained (Miller & Steinberg, 1977*b*). It is used in this paper along with the flux data to estimate the potassium current through the pump.

In the vertebrate retina, the photoreceptor outer segments and the apical surfaces of the pigment epithelial cells share the same ionic environment. They can, therefore, alter the activity of one another by causing ion or metabolite concentration changes in the space that separates them, the subretinal space. It has been shown in several different preparations (including frog) that light-evoked changes in photoreceptor activity can alter the extracellular concentration of potassium ($[K^+]_o$), which then changes the membrane potential of the pigment epithelial cells (Oakley & Green, 1976; Oakley, 1977; Matsuura, Miller & Tomita, 1978; Oakley, Flaming

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& Brown, 1979; Steinberg, Oakley & Niemeyer, 1980). These alterations in $[K]_o$ can also produce significant changes in the time course of the rod photoresponses and the transport properties of the RPE (Miller & Steinberg, 1979; Capovilla, Cervetto & Torre, 1980). The present paper shows that the range of $[K]_o$ ($[Rb]_o$) changes that stimulate active K(Rb) transport across the RPE coincides with the narrow range of $[K]_o$ changes produced by light in the subretinal space.

## Materials and Methods

These studies were performed on the isolated retinal pigment epithelium-choroid of the bullfrog *Rana catesbeiana*. The retinal pigment epithelium consists of a single layer of cuboidal epithelial cells. The basal surface of the RPE faces the choroid, which is approximately 130 µm thick and consists mainly of blood vessels and melanocytes dispersed in a fibrous stroma. Individual RPE cells measure approximately 15 µm in width and depth (Porter & Yamada, 1960; Nilsson, 1964; Steinberg, 1973). The junctional complexes connecting these cells in frogs differ from those of other species by being absent at the cellular apices; they begin at least halfway down the lateral surfaces of each cell (Porter & Yamada, 1960; Hudspeth & Yee, 1973). The apical surface of the RPE faces the sensory retina, and is covered with villous-like processes that are 60–90 µm long. These processes are closely apposed to the photoreceptors, and extend all the way down to their inner segments (Nilsson, 1964).

The bullfrogs were obtained from Californian and Midwestern suppliers, and kept from several days to several weeks in running tap water at 17.5 °C on an alternating 12-hr cycle of light and darkness. Pieces of pigment epithelium-choroid, 6.5 mm square, were isolated from dark-adapted eyes and mounted in a Lucite® chamber so that the surface of the choroid and the apical surface of the pigment epithelium were immersed in separate 1.8-ml baths whose compositions could be separately controlled. The chamber design and the techniques used for dissecting the tissue and mounting it between two Lucite plates were identical to those used previously (Miller & Steinberg, 1977*a, b*).

The composition of the control bathing solution was (in mM): 82.5 NaCl, 27.5 $NaHCO_3$, 2.0 KCl, 1.0 $MgCl_2 \cdot 6H_2O$, 1.8 $CaCl_2$, 10.0 glucose; it was gassed with 95% $O_2$/5% $CO_2$ to a pH of 7.4 ±0.1. The osmolarity of this solution was 227 mosM. In some experiments, the potassium concentration was varied by equimolar exchange of KCl and NaCl. In other experiments the concentration of potassium was increased by adding small amounts of KCl (3 mM or less) to the solutions bathing the apical or basal membranes. In control experiments as much as 3 mM Tris chloride (tris [hydroxymethyl] aminomethane hydrochloride) was added to the solutions bathing one or both sides of the tissue.

Two pairs of Agar-Ringer's bridges on each side of the tissue were used to monitor the transepithelial potential (TEP) and to pass current both for short-circuiting (short-circuit current = SCC) and for measuring the transepithelial resistance ($R_t$). SCC was monitored continuously on a pen recorder. Readings of TEP were obtained hourly by briefly interrupting the short circuit, and $R_t$ also was obtained at the same time by passing 1 µA pulses transepithelially and recording the changes in TEP.

For the determination of the transepithelial unidirectional fluxes of potassium or rubidium, a tracer amount of the isotope [$^{42}$K or $^{86}$Rb] was added either to the solution facing the choroid—the basal solution—or to the apical solution. The fluxes, apical→basal (retina→choroid) or basal→apical (choroid→retina) were then measured by sampling the "cold" solution on the

**Table 1.** Active transport (nM/cm² hr) across RPE in 2 mM K(Rb) Ringer's

| | $A \to B$ | $B \to A$ | Net flux ($A \to B$) |
|---|---|---|---|
| (*A*) Unidirectional fluxes across the RPE (nM/cm² hr)[a] | | | |
| $^{42}$K(2 mM$[K]_o$) | 86.4 ± 8.6 (7) | 47.3 ± 2.4 (7) | 39.1* |
| $R_t$(KΩ) | 4.56 ± 0.31 | 4.30 ± 0.31 | |
| SCC(µeq/cm² hr) | 1.50 ± 0.16 | 1.58 ± 0.14 | |
| $^{86}$Rb(2 mM$[Rb]_o$) | 179 ± 10.9 (27) | 33.3 ± 3.2 (15) | 145.7* |
| $R_t$(KΩ) | 4.95 ± 0.12 | 4.80 ± 0.18 | |
| SCC(µeq/cm² hr) | 1.50 ± 0.05 | 1.42 ± 0.11 | |
| $^{86}$Rb(2 mM$[K]_o$) | 94 ± 11.1 (8) | 39.5 ± 3.6 (6) | 54.5* |
| $R_t$(KΩ) | 5.4 ± 0.23 | 4.6 ± 0.49 | |
| SCC(µeq/cm² hr) | 1.40 ± 0.07 | 1.54 ± 0.15 | |
| (*B*) Tracer rate coefficients (cm² hr)$^{-1}$[b] | | | |
| $^{42}$K(2 mM$[K]_o$) | 0.024 ± 0.002 (7) | 0.013 ± 0.0007 (7) | |
| $^{86}$Rb(2 mM$[Rb]_o$) | 0.050 ± 0.003 (27) | 0.009 ± 0.0008 (15) | |
| $^{86}$Rb(2 mM$[K]_o$) | 0.026 ± 0.003 (8) | 0.011 ± 0.001 (6) | |

[a] The values of flux, $R_t$, and SCC in each column are given as the mean ± SEM. The numbers in parentheses denote the number of experiments. There were at least seven steady-state measurements per experiment and their SEM did not exceed the values shown in the Table. The net flux is in the retina-to-choroid direction ($A \to B$) and is significant (*$p < 0.001$, unpaired *t*-test). For each set of comparison experiments in columns 2 and 3 the average electrical parameters, SCC and $R_t$, were statistically indistinguishable. The Ringer's bathing both sides of the tissue contained either 2 mM $[K]_o$ (rows 1, 7) or 2 mM $[Rb]_o$ (row 4).

[b] The tracer rate coefficients for the same set of experiments as in (*A*). They can be multiplied by the amount of cold $[K]_o$ or $[Rb]_o$ in the chamber (3.6 µM) to give the unidirectional fluxes

opposite side of the tissue every 20 min. The sample size was 100 μl, and it was replaced with an equal amount of "cold" solution. Samples were assayed by conventional counting techniques, using a Beckman LS-7500 liquid scintillation spectrometer (Beckman Instruments, Inc., Mountain View, Calif.). The unidirectional fluxes were calculated in $nmol/cm^2 hr$ from the rate of tracer appearance on the "cold" side and the specific activity of the "hot" side; the area refers to the area of the window between the two Lucite chambers and is $0.07 cm^2$. The fluid in each chamber was stirred and oxygenated by a stream of water-saturated gas bubbles.

## Results

### *Potassium Fluxes and Ouabain*

RPE active transport of potassium was assessed by measuring unidirectional fluxes of $^{42}K$ across the tissue. Table 1(*A*) (row 1) summarizes the results of 14 such experiments. These were paired measurements, each pair of tissues from the same animal and frequently from the same eye. The electrical characteristics of these paired tissues were not significantly different. The retina-to-choroid ($A \rightarrow B$) flux was significantly greater than the choroid-to-retina ($B \rightarrow A$) flux ($p < 0.001$) so that there was a net flux of $39.1 nM/cm^2 hr$ in the retina-to-choroid direction. This is the *direction* of net flux expected from a Na:K pump located in the apical membrane.

The relationship between active potassium transport and $[K]_o$ was determined by measuring the ouabain-sensitive net transepithelial flux at several different levels of $[K]_o$. Figure 1 shows the potassium unidirectional fluxes when the tissue was bathed on both sides in Ringer's containing 2 mM $[K^+]_o$. These experiments were performed on paired tissues from the same eye. The closed circles represent the $A \rightarrow B$ flux and the open circles the $B \rightarrow A$ flux. The solid lines represent the average steady-state flux, and the difference between them, the net flux, is approximately $32 [nM/cm^2 hr]$. At $t = 3$ hr, $10^{-4}$ M ouabain was added to the apical bath. The unidirectional fluxes were first elevated and then decreased to their steady state 3 hr after the addition of ouabain. At this time both unidirectional fluxes were approximately $40–50 nM/cm^2 hr$. The effect of ouabain, therefore, was to bring the *net* $^{42}K$ flux to zero by reducing the unidirectional flux in the pump direction, that is, in the direction of active transport (retina to choroid). In contrast, the steady-state flux in the choroid-to-retina direction was unchanged by ouabain. This shows that the apical membrane Na:K pump plays an important role in the active transport of potassium *across* the RPE.

When $[K]_o$ was elevated from 2 to 8 mM on both sides of the tissue, the net transport rate in the retina-to-choroid direction increased by a factor of

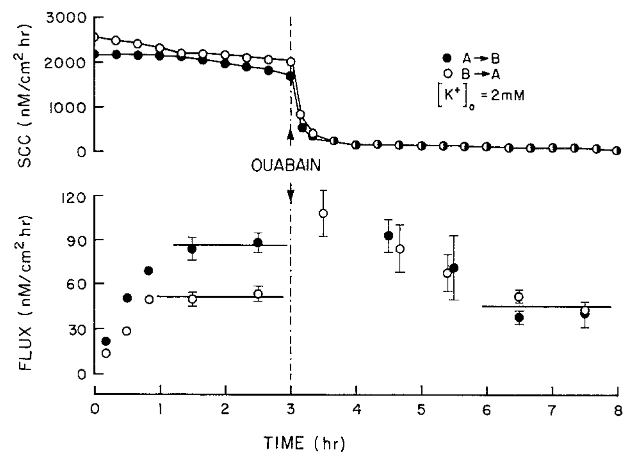


**Fig. 1.** Unidirectional $^{42}K$ fluxes with 2 mM $[K]_o$ in both solutions. Tracer, $^{42}K$, was added either to the apical solution (closed symbols) or the basal solution (open symbols) at $t = 0$, and the level of radioactivity was sampled at 20-min intervals from the "cold" solution. The open symbols represent the choroid-to-retina unidirectional fluxes and the closed symbols represent the retina-to-choroid unidirectional fluxes, the direction of active transport. Both tissues were from the same eye. Prior to the addition of ouabain, the average values of $R_t$ and TEP were 4.5 kΩ and 17 mV, respectively, for the retina-to-choroid flux and 4.4 kΩ and 18 mV for the choroid-to-retina flux. Ouabain, $10^{-4}$ M, was added to the apical solution at $t = 3$ hr and caused a large drop in TEP and a significant increase in $R_t$. Within 20 min, $R_t$ increased from 4.5 kΩ to 4.9 kΩ in the $A \rightarrow B$ experiment and from 4.4 kΩ to 5.3 kΩ in the $B \rightarrow A$ experiment. The increases in $R_t$ continued on for another hour and then leveled off at $4\frac{1}{2}$ hr to approximately 5.5 kΩ ($A \rightarrow B$) and 6.0 kΩ ($B \rightarrow A$). The closed symbols at $t = 3\frac{1}{2}$ hr has been omitted for the sake of convenience; it is $217 \pm 23 [nM/cm^2 hr]$. In this Figure and in Figs. 2–4, the open and closed symbols with error bars represent the mean ± SEM of three consecutive 20-min samples

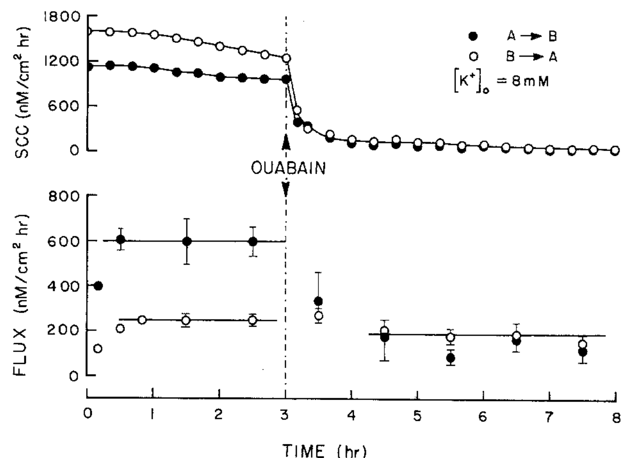


**Fig. 2.** Unidirectional $^{42}K$ fluxes with 8 mM $[K]_o$ in both solutions. Otherwise as Fig. 1. In the $A \rightarrow B$ experiment, the average value of TEP and $R_t$ before the addition of ouabain was 10 mV and 4.2 kΩ, respectively. These parameters were 11.5 mV and 3.8 kΩ in the $B \rightarrow A$ experiments. After the addition of ouabain, $R_t$ rose continuously for 2 hr from 4.2 to 7.2 kΩ and then levelled off at 7.2 kΩ ($A \rightarrow B$ experiment). In the $B \rightarrow A$ experiment, $R_t$ rose over the first $1\frac{1}{2}$ hr after ouabain from 3.8 to 5.5 kΩ and then levelled off at 5.5 kΩ

approximately 10 to 350 [nM/cm$^2$ hr] (Fig. 2). Compared to the fluxes in 2 mM $[K]_o$ (Fig. 1), both unidirectional fluxes were increased, but the flux in the choroid-to-retina direction increased by a much smaller amount. In both of the experiments in Fig. 2, ouabain, $10^{-4}$ M, was added to the apical solution at $t=3$ hr. Both fluxes reached a steady state value of 200 (nM/cm$^2$ hr) 1 hr *after* the addition of ouabain. As in 2 mM $[K]_o$ (Fig. 1), ouabain reduced the *net* transepithelial flux to zero and the entire reduction was due to a decrease of the flux in the pump direction.

Figure 3 illustrates the $^{42}$K unidirectional fluxes in another set of paired experiments in which the Ringer's bathing the tissues contained only 0.42 mM $[K^+]_o$. In these experiments the net flux prior to the addition of ouabain was approximately 9.3 [nM/cm$^2$ hr]. Again, ouabain had no effect on the steady-state choroid-to-retina ($B \to A$) flux but decreased the retina-to-choroid ($A \to B$) flux from 19 [nM/cm$^2$ hr] to 10 [nM/cm$^2$ hr], thereby reducing the net active $^{42}$K flux to zero. One difference in 0.42 mM $[K]_o$ is the low level of SCC compared to 2.0 mM $[K]_o$ (Fig. 1) and 8.0 mM $[K]_o$ (Fig. 2). This is because the Na:K pump is electrogenic and sets up a significant portion of the transepithelial potential, which is reduced by bathing the tissue in low $[K^+]_o$ Ringer's (Miller et al., 1978; Oakley et al., 1978). The average value of TEP prior to the addition of ouabain was only 2.9 mV in the $A \to B$ experiment and 2 mV in the $B \to A$ experiment. After the addition of ouabain, the TEP declined in both cases to 0 mV and then slightly rebounded. In both experiments there was no significant change in transepithelial resistance after the addition of ouabain (*see* Fig. 3 legend).

In sum, the data in Table 1(*A*) and Figs. 1, 2 and 3 show that $^{42}$K is actively transported across the RPE in the retina-to-choroid direction, that this net flux increases with $[K]_o$ and that at each level it is completely inhibited by apical ouabain.

### *Ouabain-Induced Transients*

Figure 1 shows that after ouabain there is a transient increase in unidirectional flux followed by a decrease to the steady state (solid lines). Since each data point is the mean ±SEM of three consecutive 20-min samples, the initial increase was partially obscured by averaging over the first hour. For example, in the $A \to B$ experiment, the first data point at 20 min was 240 [nM/cm$^2$ hr] which is more than twice the pre-ouabain steady-state flux. In the $B \to A$ experiment the initial flux after ouabain was 130 [nM/cm$^2$ hr], also a factor of 2 greater than the pre-ouabain steady-state flux. These increases after ouabain were observed, without exception, in a dozen tissues bathed in 2 mM $[K]_o$. In 8 mM $[K]_o$ (Fig. 2) and 0.42 mM $[K]_o$ (Fig. 3), ouabain also produced a transient increase in the first flux samples (obscured here by the 1-hr averaging), but they were never more than 30% greater than the pre-ouabain steady-state levels. The magnitude and time course of these transients as a function of $[K]_o$ are difficult to predict because they depend on the K electrochemical gradients at the apical and basal membranes, the contribution of the electrogenic Na:K pump to membrane potential, the shunt and cell membrane conductances and the time course of K uptake and release from the intracellular compartments of the choroid (Miller & Steinberg, 1977*b*; Miller et al., 1978).[1] These ouabain-induced $^{42}$K transients have also been observed by Wright (1972) in the frog choroid plexus.

### *Rubidium Fluxes*

Since the $^{42}$K isotope is relatively inconvenient to use (half-life of 12.4 hr and expensive), we continued these experiments using $^{86}$Rb, which has a half-life of approximately 18.7 days. Figure 4 illustrates the effect of ouabain on the unidirectional $^{86}$Rb fluxes. Paired tissues from the same eye were bathed on both sides with 2 mM $[Rb]_o$ Ringer's and prior to the addition of ouabain the net flux was 170 (nM/cm$^2$ hr). Ouabain, $10^{-4}$ M, reduced the net

[1] The transient in the $B \to A$ direction probably results from an increase in the passive leak of $^{42}$K down its electrochemical gradient into the apical solution. This would occur because ouabain shut off the electrogenic sodium pump, significantly depolarized the apical membrane potential (Miller et al., 1978), and increased the K electrochemical gradient. Eventually, as the K electrochemical gradient ran down, cell K would be depleted and the net efflux across the apical membrane decreased until it was matched, in the steady state (solid lines, Fig. 1), by the net influx across the basal membrane. One test of this mechanism would be to block the potassium channels in the apical membrane, in which case one would expect a reduction in the size of the transient. It has been shown in nerve, muscle and epithelial tissues that barium blocks K channels (Hermsmeyer & Sperelakis, 1970; Henderson, 1974; Nagel, 1979; Nielson, 1979). In the RPE, 1 mM barium in the apical solution decreased the apical membrane K conductance and completely removed the $B \to A$ transient (S.S. Miller, *in preparation*).

The transient in the $A \to B$ flux is probably initiated by a reduction in the amount of potassium ($^{42}$K) flowing across the basal membrane into the choroid. This would lower the extracellular K concentration in the choroid and trigger a release of K from the choroidal stores into the surrounding extracellular space. Previous experiments with $K^+$-specific microelectrodes indicated that there was a significant amount of uptake or release of $K^+$ from the intracellular compartments of the choroid (Miller & Steinberg, 1977*b*). In support of this hypothesis is the observation that 1 mM barium, in the basal solution, significantly reduced the $A \to B$ transient without affecting the basal membrane potential.

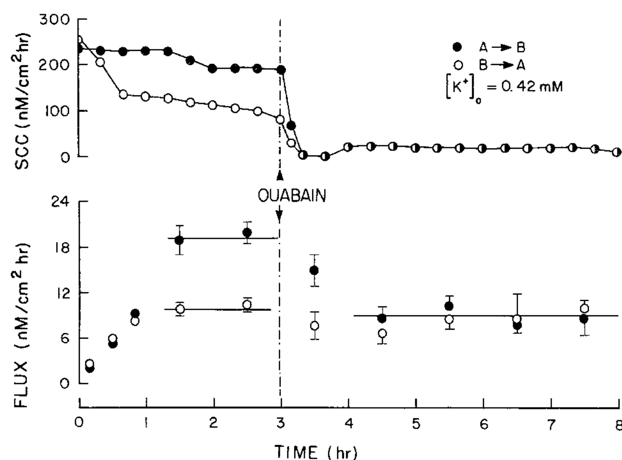


**Fig. 3.** Unidirectional $^{42}K$ fluxes with 0.42 mM $[K]_o$ in both solutions. Otherwise as Fig. 1. In the $A \rightarrow B$ experiment, $R_t$ was 5.5 kΩ in the last hour before the addition of ouabain. This resistance remained constant over the next 5 hr. In the $B \rightarrow A$ experiment, $R_t$ was 5.2 kΩ prior to the addition of ouabain and then decreased over the next 6 hr to 3.4 kΩ

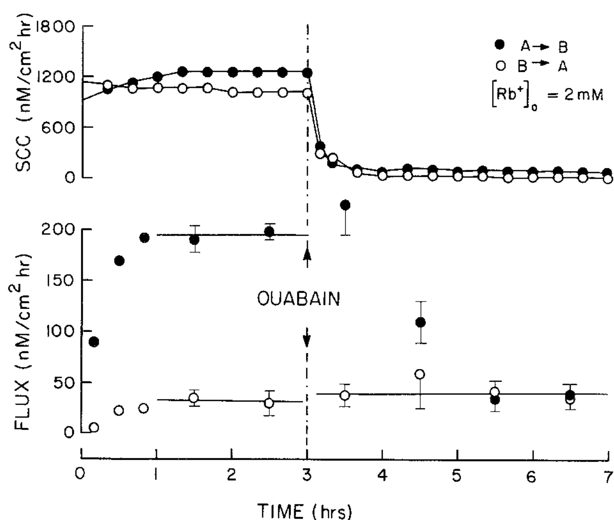


**Fig. 4.** Unidirectional $^{86}Rb$ fluxes with 2 mM $[Rb]_o$ in both solutions. Otherwise as Fig. 1. In the $A \rightarrow B$ experiment, the average value of TEP and $R_t$ before the addition of ouabain was 12 mV and 5.2 kΩ, respectively. These parameters were 7.9 mV and 4.3 kΩ in the $B \rightarrow A$ experiment. In the first 20 min after the addition of ouabain, the TEP in both cases fell to approximately 2.0 mV and then slowly continued to decrease over the next 4 hr toward 0 mV. In the $A \rightarrow B$ experiment, $R_t$ rose continuously from 5.2 to 6.1 kΩ. In the $B \rightarrow A$ experiment, $R_t$ rose from 4.3 to 4.7 kΩ in the first hour after the addition of ouabain and then remained constant

active transport, in the steady state, to zero without altering the $B \rightarrow A$ flux. In this case the $A \rightarrow B$ flux was reduced by more than a factor of 5 from approximately 200 (nM/cm² hr) to 35 (nM/cm² hr). This result, along with those in Figs. 1–3, helps to show that ouabain has the same qualitative effect on the unidirectional fluxes whether the cation in the external solution is potassium or rubidium.

Table 1(*A*), rows 1 and 4, summarizes the data from a large number of experiments in which the tissue was bathed on both sides with 2 mM $[K]_o$ or 2 mM $[Rb]_o$ Ringer's. Row 4 shows that the average net $^{86}Rb$ flux across the RPE is 145 (nM/cm² hr), approximately a factor of 3.6 larger than the net $^{42}K$ flux (row 1). This difference in net flux is almost entirely due to the large difference in the $A \rightarrow B$ fluxes. It cannot be explained by the difference in transepithelial resistance (rows 2 and 5) because the difference is small and in the wrong direction.

In the $B \rightarrow A$ experiments (rows 1 and 4) the average transepithelial resistance was also slightly larger in $[Rb]_o$, as compared to $[K]_o$ Ringer's (4.80 ±0.18 kΩ versus 4.30±0.30 kΩ). This difference might be due to a selectivity difference in the passive transport pathways through the cell and/or the shunt or it might be caused by a somewhat tighter mechanical seal around the edge of the tissue in the Rb experiments. In any case, the average $B \rightarrow A$ flux was larger ($p < 0.005$) by 14 (nM/cm² hr) in $[K]_o$ Ringer's.

### *$[K]_o$-, $[Rb]_o$-Dependent $^{86}Rb$ Transport*

The effects of 2 mM $[Rb]_o$ and 2 mM $[K]_o$ on the transepithelial movement of $^{42}K$ and $^{86}Rb$ were compared using tracer rate coefficients [Table 1(*B*)]. These coefficients, which have units of $(cm^2 hr)^{-1}$, are defined as the unidirectional tracer flux per total amount of tracer on the "hot" side (Dawson, 1977). The unidirectional fluxes in Table 1*A*, which have units of nM/cm² hr, are obtained by multiplying the rate coefficients by the amount of abundant species (2 mM × 1.8 ml or 3.6 μM). In the steady state the rate coefficients are constant and therefore provide a phenomenological characterization of the transport pathway.

When the tissue is bathed in 2 mM $[K]_o$ Ringer's (rows 1 and 3), the rate coefficients for $^{42}K$ and $^{86}Rb$ tracer movement are practically identical (columns 2 and 3). Since the tracer places such a small perturbation on the system, this strongly suggests that $^{86}Rb$ can be used as a substitute for $^{42}K$ in tracing the movement of potassium across the RPE. The unidirectional fluxes of $^{86}Rb$, shown in row 7 of Table 1(*A*), were calculated on the basis of this assumption.

The stimulation of net epithelial transport was next studied as a function of $[K]_o$ and $[Rb]_o$ concentration (0.2 to 10 mM). The data from 65 experiments are summarized in Fig. 5, where the solid lines connect the fluxes obtained in potassium Ringer's and the dashed lines connect the fluxes obtained in rubidium Ringer's. Two different types

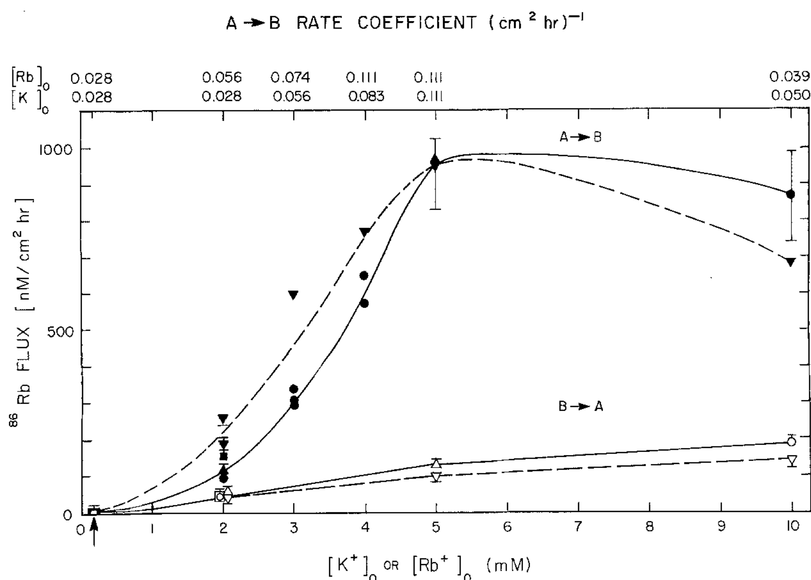


**Fig. 5.** Unidirectional ${}^{86}$Rb fluxes as a function of extracellular potassium or rubidium. The filled symbols represent the retina-to-choroid fluxes ($A \rightarrow B$) and the open symbols choroid-to-retina fluxes ($B \rightarrow A$). The lines best fitting the data were estimated by eye; solid lines connect the fluxes obtained in potassium Ringer's and interrupted lines connect the fluxes obtained in rubidium Ringer's. Each data point without an error bar is the mean steady-state unidirectional flux from one tissue. There are at least seven steady-state measurements per point, and the SEM in each case is no greater than the size of the data point. The potassium or rubidium concentration was constant on both sides of the tissue throughout some of the experiments at 2 mM and all of the experiments at 10 mM (●, ○; ▼, ▽). In all other experiments, the steady-state unidirectional fluxes were determined for each tissue at two different concentrations. These kinds of experiments were done between 0.2 and 2.0 mM (■, □), between 2 and 3, or 4 mM (●, ○, ●, ▼, ▽) and between 2 and 5 mM (▲, △, ▼, ▽). The experiments represented by the data points with error bars (mean ± SEM) consist of steady-state measurements on five or more tissues. The average value of the tracer rate coefficients (retina-to-choroid) are compared at several concentrations of $[K]_o$ and $[Rb]_o$ (top of Figure). These average values were calculated at each concentration using the unidirectional flux given by the solid and dotted lines

of experiments were done. In the first type the concentration of $[K^+]_o$ or $[Rb^+]_o$ was maintained constant throughout the experiment. The open and closed circles at 2 mM (replotted from Table 1) and 10 mM represent experiments of this type. The squares and the triangles at 2 mM and the closed symbols at 3, 4 and 5 mM are examples of the second type of experiment in which the tissues were first bathed on both sides with one concentration and then, after 2 hr in the steady state, the concentration of $[K^+]_o$ (or $[Rb^+]_o$) was elevated on both sides of the tissue and the experiment continued for another $2\frac{1}{2}$ hr.[2] In this second type of experiment the tissues were *initially* bathed in 0.2 mM Ringer's ($n = 10$, arrow on left) or 2 mM Ringer's ($n = 16$).

Figure 5 (dashed lines) shows that the *net* ${}^{86}$Rb flux in $[Rb]_o$ Ringer's increased monotonically between 0.2 and 5 mM and then decreased slightly between 5 and 10 mM. A similar relationship was obtained using $[K]_o$ Ringer's and ${}^{86}$Rb (solid lines).[3] Between 2 and 5 mM in the ${}^{86}$Rb flux is greater in $[Rb]_o$ than in $[K]_o$ Ringer's. At 2 mM the difference is approximately 90 [nM/cm² hr] and at 3 mM the difference is approximately 150 [nM/cm² hr]. At 5 mM the maximum rate of active transport is practically the same in potassium and rubidium Ringer's. These differences result mainly from an increases in the $A \rightarrow B$ flux since the $B \rightarrow A$ unidirectional fluxes are nearly identical over this range of $[Rb]_o$ and $[K]_o$ changes.

Although the rate coefficients are invariant with respect to isotope (${}^{42}$K or ${}^{86}$Rb), they are not invariant with respect to the concentration of the abundant species ($[K]_o$ or $[Rb]_o$). This is illustrated at the top of Fig. 5, which shows that increasing the concentration of $[K]_o$ or $[Rb]_o$ between 0.2 and 5 mM caused a fourfold increase in the $A \rightarrow B$ rate coefficient. It is also clear that the rate coefficients

[2] The observed changes in net flux with concentration are specific to the cations involved and are not osmotic in origin. Adding 1.8 or 3 mM Tris Cl to the solutions bathing one or both sides of the tissue produced little change in either of the unidirectional fluxes (6 experiments). The observed increases in net flux were also obtained when $[K^+]_o$ was elevated only on the apical side of the tissue (4 experiments). Increasing basal $[K^+]_o$ had no effect on either of the unidirectional fluxes (4 experiments).

[3] Again, it wa assumed that ${}^{86}$Rb could be used as a substitute for ${}^{42}$K in calculating the unidirectional fluxes. This assumption is supported by the near equivalence of the ${}^{86}$Rb (Fig. 5) and ${}^{42}$K rate coefficients at $[K]_o = 0.42$, 2 and 8 mM (*see* Table 1(*B*) and Figs. 1, 2 and 3).

were significantly larger in $[Rb]_o$ as compared to $[K]_o$ Ringer's (*see also*, Table 1(*B*), rows 1 and 2). In contrast, the rate coefficients for tracer movement in the $B \rightarrow A$ direction are relatively independent of concentration *and* composition ($[K]_o$ or $[Rb]_o$). They only varied from 0.010 to 0.014 $(cm^2 \, hr)^{-1}$ across the entire range of concentrations.

Since most membranes can discriminate to some extent between $[K]_o$ and $[Rb]_o$ (Sjodin, 1959; Moore, Anderson, Blaustein, Takata, Lettvin, Pickard, Bernstein & Pooler, 1967; Standen & Stanfield, 1980), the latter result suggests that most of the $B \rightarrow A$ flux transverses the shunt pathway. The large differences in the $A \rightarrow B$ rate coefficients could have occurred because the active transport mechanism in the cellular pathway discriminate between Rb and K or because extracellular rubidium can block the passive K channels (*see* Appendix). In a recent voltage-clamp study of frog skeletal muscle, it was demonstrated that $[Rb]_o$ blocked the K channels and reduced membrane conductance (Standen & Stanfield, 1980). It should be emphasized that under *identical* conditions the tissue *does not* discriminate between $^{42}K$ and $^{86}Rb$ but that extracellular (abundant) Rb is a more potent "activator" of the active flux due to increased activation of (Na, K)-ATPase or selective blockade of membrane channels.

### *$[K]_o$-Dependent $^{22}Na$ Transport*

Previously, we showed that the RPE actively transports Na in the choroid-to-retina direction and that this net transport, $\simeq 470 \, (nM/cm^2 \, hr)$, is significantly inhibited by $10^{-4}$ M ouabain, in the apical solution (Miller & Steinberg, 1977*a*). When ouabain is placed in the solution bathing the apical membrane, it affects the net $^{22}Na$ flux and depolarizes the apical membrane (Miller et al., 1978), but when it is placed in the solution bathing the basal membrane, it has no effect on membrane potential or undirectional flux (Steinberg & Miller, 1973). In all systems the direction of Na transport is normally toward the extracellular side of the plasma membrane that contains the (Na, K)-ATPase and, since ouabain inhibits transport only when added to the apical side, this strongly suggests that the apical membrane of the RPE contains the (Na, K)-ATPase. This conclusion was corroborated by experiments that localized the ATPase to the apical membrane (Bok, 1980; Ostwald & Steinberg, 1980).

If the apical membrane Na:K pump is responsible for transepithelial sodium transport, then it should be possible to alter $[K]_o$ and affect the net rate of $^{22}Na$ movement across the RPE. The protocol of these experiments was to first measure the undirectional $^{22}Na$ fluxes, $A \rightarrow B$ or $B \rightarrow A$, in 2 mM $[K]_o$ Ringer's and then, after 2 hr in the steady state, the $[KCl]_o$ concentration on both sides of the tissue was increased by 3 mM and the measurements continued for another 3 hr. In control experiments on four tissues, adding 3 mM Tris Cl to both sides had little effect on TEP, $R_t$, or undirectional $^{22}Na$ flux (*see also*, footnote 3). The undirectional fluxes in the $A \rightarrow B$ direction ($n=5$) increased by an average of $50 \, (nM/cm^2 \, hr)$, but the difference is not significant ($p > 0.2$). In 2 out of 5 of these experiments there was *no* change in flux. The $B \rightarrow A$ fluxes increased by an average of $370 \, (nM/cm^2 \, hr)$ ($p=0.007$, $n=5$) and the smallest increase was $230 \, (nM/cm^2 \, hr)$. Therefore, elevating $[K]_o$ from 2 to 5 mM increased the net $^{22}Na$ transport by $\simeq 300 \, (nM/cm^2 \, hr)$.

In both sets of experiments, $A \rightarrow B$ and $B \rightarrow A$, elevating $[K]_o$ decreased the average transepithelial resistance and the effect was somewhat larger in the $A \rightarrow B$ ($4.0 \pm 0.17 \, k\Omega \rightarrow 3.5 \pm 0.23 \, k\Omega$) than the $B \rightarrow A$ ($4.5 \pm 0.53 \, k\Omega \rightarrow 4.2 \pm 0.73 \, k\Omega$) experiments. This slight decrease in $R_t$ cannot explain the increase in net flux since there were two $B \rightarrow A$ experiments in which $R_t$ was either constant or increased (the flux increase was $230 \, (nM/cm^2 \, hr)$ and $340 \, (nM/cm^2 \, hr)$, respectively). In most cases, $[K]_o$ was elevated on both sides of the tissue in order to maintain the transepithelial electrochemical gradient at zero. However, several experiments had $[K]_o$ increased on the apical side only (3 mM Tris Cl on the basal side) and this produced the same alterations in TEP, $R_t$, and flux as the both-sided changes. Since the Na:K pumps are located on the apical membrane, this suggests that the change in net flux was generated by the change in apical solution $[K]_o$.

## Discussion

The retinal pigment epithelium actively transports potassium (rubidium) from the retinal-to-choroidal side of the tissue. The present study shows that: (a) this net transport is completely inhibited by $10^{-4}$ M ouabain in the solution bathing the apical membrane; (b) ouabain inhibits the unidirectional flux in the pump direction and has no effect on the "passive flux" in the choroid-to-retina direction; (c) the active transport traced by $^{42}K$ or $^{86}Rb$ increased monotonically as $[K]_o$ or $[Rb]_o$ was increased from 0.2 to 5 mM; (d) the ouabain-sensitive net flux is greater in Rb than in K Ringer's while the maximum transport rates are the same for both cations; (e) increasing $[K]_o$ from 2 to 5 mM also stimulated net epithelial Na transport.

**Table 2.** Sodium and potassium pump current[a]

| $[K]_o$ (mM) | Tracer | $I_{SCC}$ (nM/hr) | $I_{Leak}$ (nM/hr) | $I_{Pump}$ (nM/hr) | $I_{Na}^{Pump}/I_K^{Pump}$ |
|---|---|---|---|---|---|
| 2 | $^{22}Na$ | 33 | 63–86 | 96–116 | $\simeq$1.0–2.0 |
| | $^{42}K$ | 3 | 53–106 | 56–109 | |
| 5 | $^{22}Na$ | 54 | 60–78 | 114–132 | $\simeq$1.1–2.4 |
| | $^{86}Rb$ | 56 | 0–44 | 56–100 | |

[a] The data in column 3, $I_{SCC}$, summarize the mean net epithelial flux obtained in this study (rows 3, 4 and 5) and in a previous study (row 2; Miller & Steinberg, 1977*a*). The values of leak current in column 4 were almost entirely obtained from electrophysiology data on the RPE (Miller & Steinberg, 1977*b*; Oakley et al., 1978). Because $E_{Na}$ was not measured in the RPE, a range of values for $[Na]_i$ was assumed from ion-specific microelectrode measurements in several other epithelia (*see text* for references). The variability in the transport data was 10–20% of the variability in the electrophysiological results, and therefore no range of values is shown in column 3. In 2 mM $[K]_o$ Ringer's the average value of $V_m$ in the short-circuited state (−84 mV) was calculated for the electrophysiologically derived equivalent circuit of the RPE (Miller & Steinberg, 1977*b*). This calculation depended on the average TEP (10–12 mV), cell membrane and shunt resistances and membrane potential (−88 mV; obtained from more than 500 cells and 150 tissues with 85% of the cells between −75 and −95 mV) (Miller & Steinberg, 1977*b*; Oakley et al., 1978). The best estimate of relative sodium conductance is 5%, but the experimental data does not allow one to distinguish clearly between 5 and 10%. The latter value corresponds to a change in apical membrane potential of 3–4 mV for a 10-fold change in sodium concentration.

### *Transepithelial Potassium and Sodium Transport and the Na:K Pump*

In red blood cells, nerve, muscle and epithelial tissues it has been shown that extracellular *and* intracellular potassium are both important determinants of Na:K pump activity (Garay & Garrahan, 1973; Knight & Welt, 1974; Kennedy & DeWeer, 1977*b*; Abercrombie & DeWeer, 1978; Cala, Cogswell & Mandel, 1978). In these systems the coupling ratio (Na pump current/K pump current) is normally greater than 1; that is, the pump current contributes directly to membrane potential (Thomas, 1972; Hoffman, Kaplan & Callahan, 1979; Nielsen, 1979). In the RPE the apical membrane sodium pump is also electrogenic, and it has been shown that the contribution of this pump to apical membrane potential can be altered by lowering apical $[K]_o$ or elevating $[K]_i$ (Miller et al., 1978; Oakley et al., 1978). The data in the present paper plus previous electrophysiological and transport data (Miller & Steinberg, 1977*a*, *b*) allowed us to estimate the K and Na pump current and coupling ratio at two different levels of $[K]_o$. The details of the analysis are given in the Appendix and the overall results are summarized in Table 2. This analysis permits a comparison of the *pump* flux-ratio and the transepithelial flux-ratio. For example, at $[K]_o = 2$ mM the Na, K flux-ratio through the pump is approximately 3/2 while the Na, K net transepithelial flux-ratio is 11/1. The net $^{42}K$ flux across the RPE in 2 mM $[K]_o$ Ringer's is 3 nM/hr, while the estimated K flux through the pump is 56–109 nM/hr. This large difference is not surprising given the substantial potassium current that moves through the passive K channels in the apical and basal membranes of the RPE (Miller & Steinberg, 1977*b*; Oakley et al., 1978).

### *$[K]_o$ in the Dark and in the Light*

The extracellular space that surrounds the photoreceptor outer segments is called the subretinal space. It is bounded distally by the apical membranes of the RPE cells and proximally by the Müller cells and basal portions of the photoreceptor inner segments. Sharing this space with the photoreceptors are the apical processes of the RPE cells (*see* Materials and Methods) and the villous processes of the Müller cells. In the dark-adapted vertebrate retina, the concentration of potassium in the subretinal space is relatively high and is reduced by light (Oakley & Green, 1976; Oakley, 1977; Matsuura et al., 1978; Oakley et al., 1979; Steinberg et al., 1980). It has been suggested that the light-evoked hyperpolarization of the photoreceptor membrane reduces the net efflux of K from the photoreceptors and that this reduction is mainly responsible for the observed decrease of $[K]_o$ in the subretinal space. All of the available data is consistent with this hypothesis (Cavaggioni, Sorbi & Turini, 1973; Matsuura et al., 1978; Oakley et al., 1979).

The data in the present paper allow us to determine if transport across the RPE is capable of modifying the light-evoked $[K]_o$ changes in the subretinal space. For example, in the intact cat eye (Steinberg et al., 1980) and in the isolated frog retina-PE preparation (Oakley & Steinberg, 1981) long steps of light (10 min) decrease $[K]_o$ in the subretinal space. In both cases there is a subsequent rebound to a new steady state. In the frog the initial decrease, from 3.3 to 1 mM, is probably due to the light-induced hyperpolarization of the photoreceptors, but the rebound, from 1 to 1.8 mM, cannot be explained on these grounds since the photoreceptors remain hyperpolarized in the light (Lipton, Ostroy & Dowling, 1977). This rebound occurred in $\simeq$7 min and might be caused, in part, by a decrease of net K transport across the RPE.

Figure 5 shows that $^{86}Rb$ transport is signifi-

cantly reduced when $[K]_o$ is decreased from 3.3 to 1 mM. The difference in net transport rate between 3.3 and 1 to 1.8 mM is $\simeq 240\,\text{nM/cm}^2\,\text{hr}$, and since the area of tissue is $0.07\,\text{cm}^2$, the isolated RPE predicts an accumulation rate of $\simeq 0.3$ nM/min. This is a factor of 16 larger than the accumulation predicted from the retina-PE preparation even if one assumes that the extracellular fraction on the subretinal volume ($0.07\,\text{cm}^2 \times 0.01$ cm) is as large as 10% (Faber, 1969; Ogden & Ito, 1971).

The flux data from short-circuited RPE's would be expected to overestimate the accumulation rate obtained in the *in-vitro* retina-RPE preparation which was *not* voltage-clamped to zero. Therefore additional flux measurements were made under open-circuit conditions (TEP = 12.1 ± 0.9 mV, mean ± SEM, $n=6$; *data not shown*). These data predict an accumulation rate of $\simeq 0.2$ nM/min, still a factor of 10 larger than observed in the retina-RPE preparation. This discrepancy might be reduced further if there were other substances in the subretinal space like taurine (Miller & Steinberg, 1979) that could affect RPE potassium transport. In any case, these calculations show that the RPE transport systems have more than sufficient capacity to contribute to the slow increase in $[K]_o$.

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## Appendix

The following analysis assumes that potassium moves across the apical membrane of the RPE by two mechanisms, the Na:K pump and an electrochemical driving force in the "passive" K channels. On the basal membrane there is only evidence for the latter mechanism (Introduction). The calculations were carried out by using a relationship derived by Ginzburg and Hogg (1967). This relationship is obtained from an equivalent circuit that is appropriate for the RPE (Miller & Steinberg, 1977*b*; Miller et al., 1978). In the steady state,

$$I_{SCC}^{K} = I_{K}^{Pump} - g_K(V_m - E_K) = g'_K(V_m - E_K) \quad (1)$$

where $I_{SCC}^{K}$ is the net potassium transport across the tissue in the short-circuited state and $I_K^{Pump}$ is the potassium that moves into the cells from the apical solution via the pump. The net K current that is electrochemically driven out of the cells through the apical and basal membranes is given by $g_K(V_m - E_K)$ and $g'_K(V_m - E_K)$, respectively. The K conductances of these membranes are $g_K$ (apical) and $g'_K$ (basal). The above relationship assumes that the tissue is short-circuited and bathed on both sides with identical Ringer's. Therefore, the driving force across *both* membranes is $V_m - E_K$. The above relation ignores nonconductive exchange mechanisms and K:K or Na:Na exchange by the pump, which should be negligible under normal metabolic conditions (Simons, 1974; Kennedy & DeWeer, 1977*a*, *b*). A similar relationship holds for sodium.

### *Pump Current in 2 mM $[K]_o$ Ringer's*

In 2 mM $[K]_o$ Ringer's, the net Na transport across the tissue is $I_{SCC}^{Na} = 470 \pm 50\,[\text{nM/cm}^2\,\text{hr}]$ (mean ± SEM, $n=20$; Miller & Steinberg, 1977*a*, Table 1) and this corresponds to a current of 33 nM/hr since the geometrical area of the tissue is $0.07\,\text{cm}^2$. The apical membrane sodium conductance, $g_{Na} \simeq 1.4 \times 10^{-5}$ mho, was calculated from the relation $T_{Na} = g_{Na} R_A$, where the relative sodium conductance is $T_{Na} \simeq 0.05$ and the total apical membrane resistance is $R_A \simeq 3.5\,\text{k}\Omega$ (Miller & Steinberg, 1977*b*, Table 1). Based on the data obtained in a wide variety of other epithelia, we assume for the RPE that $[Na]_i$ lies between 7 and 30 mM (Rick, Dorge, Von Arnim & Thurau, 1978; Zeuthen, 1978; Armstrong, Garcia-Diaz, O'Dougherty & O'Regan, 1979; Lewis & Wills, 1979; Reuss & Weinman, 1979; Spring & Kimura, 1979). In the short-circuited RPE, $V_m \simeq -84$ mV (inside negative). This estimate from intracellular recordings in the RPE was also derived from previous electrophysiological data (*see* legend to Table 2). The limits for $[Na]_i$ allow one to estimate the driving force across the Na channels, $V_m - E_{Na} \simeq 117 - 154$ mV, and multiplication by $g_{Na}$ gives $I_{Na}^{Leak} \simeq 63$–83 (nM/hr). Therefore, using Eq. (1), the Na pump current is estimated to be 96–116 (nM/hr).

In 2 mM $[K]_o$ Ringer's, the net potassium flux is approximately 40 $[\text{nM/cm}^2\,\text{hr}]$ (Table 1), a factor of 10 smaller than the net Na flux. This is not surprising in view of the relatively large passive leakage of K from the cell into the apical bath. Relative to Na the apical membrane potassium conductance is quite large, $T_K \simeq 0.52$, but the passive driving force is significantly smaller, $V_m - E_k \simeq 10$–20 mV (Miller & Steinberg, 1977*b*; Oakley et al., 1978; *see* legend to Table 2). Consequently, the K pump current is estimated to be 56–109 (nM/hr).

These calculations, based on electrophysiological and flux data, independently confirm what was previously demonstrated, which is that the Na pump is electrogenic (Miller et al., 1978). The coupling ratio in 2 mM $[K]_o$ Ringer's is $I_{Na}^{Pump}/I_K^{Pump} \simeq 1.0 - 2.0$.

In the steady state, under short-circuit current conditions, the net flux of Na across the tissue must equal the net flux across the apical and basal membranes [Eq. (1)]. We previously showed that the total basal membrane reistance $R_B$ is $\simeq 6.5\,\text{k}\Omega$ and that $T_{Na} \simeq 0.05$ at the basal membrane (Miller & Steinberg, 1977*b*). This allowed us to calculate $g'_{Na}$ [*see* Eq. (1)] and the passive leak of Na into the cell from the basal solution, $I_{Na}^{Leak} \simeq 37$ [nM/hr]. This calculation from electrophysiological data agrees very well with the net $^{22}$Na transport rate, 33 nM/hr. In the case of potassium the net flux across the tissue and across the basal membrane is $\simeq 3$ [nM/hr]. Since the driving force is 10 to 20 mV, this implies [Eq. (1)] that the relative potassium conductance at the basal membrane is quite low, $T_K \simeq 0.05$, for *outward* K movements. This is consistent with our previous finding that $T_K$ is small, on the order of 0.05 when $[K]_o$ is *decreased* from 2.0 to 0.2 mM. When basal $[K]_o$ is increased, $T_K$ is much larger (Miller & Steinberg, 1977*b*), suggesting that the basal membrane K channels are rectifying and have a much smaller conductance in the outward direction (Hodgkin & Horowicz, 1959).

### *Pump Current in 5 mM $[K]_o$ Ringer's*

If the sodium transport mechanism is similar to those studied in red blood cells, nerve and muscle, then elevating $[K]_o$ should increase both the Na and K pump current. Increasing $[K]_o$ from 2 to 5 mM on both sides of the tissue increased the *net* $^{22}$Na flux in the choroid to retina ($B \rightarrow A$) direction to about 770 $(\text{nM/cm}^2\,\text{hr})$ or 54 (nM/hr). Elevating $[K]_o$ depolarized the apical membrane by $\simeq 10$ mV (Miller & Steinberg, 1977*b*, *see* Eq. (10) and Figs. 9 and 11) and reduced the driving force across the Na channels by a similar amount assuming that $E_{Na}$ remained approximately con-

stant. Apical membrane sodium was approximately constant since small elevations in $[K]_o$ have no observable effect on membrane conductance (Miller & Steinberg, 1977*b*). From Eq. (1), $I_{Na}^{Pump} \simeq 114$–$132$(nM/hr).

At $[K]_o = 5$ mM the net transport of $^{86}$Rb was 800 [nM/cm$^2$ hr] (Fig. 5) or $I_K^{SCC} \simeq 56$ [nM/hr]. This small increase in $[K]_o$ did not appreciably alter $g_K$ or $[K]_i$; $V_m - E_K$ was decreased to less than 10 mV by increasing $[K]_o$, and changes in pump electrogenicity could be ignored (Miller & Steinberg, 1977*b*; Oakley et al., 1978). From Eq. (1), $I_K^{Pump} \simeq 56$–$100$ [nM/hr].

In sum, increasing $[K]_o$ from 2 to 5 mM increased the Na pump current by approximately 16 nM/hr,[4] but the increase in K pump current could not be quantified due to the uncertainty in the estimates of $V_m$ and $E_K$. The K pump current must have increased since the ouabain-inhibited net potassium transport increased with increasing $[K]_o$ (*compare* Figs. 1, 2 and 3). It should be clear that the estimates of K leak current are very sensitive to the uncertainties in the magnitude of $V_m - E_K$ because the upper and lower bounds, 20 and 10 mV (at 2 mM $[K]_o$), respectively, are approximately, equal to their difference (10 mV). The estimates of Na leak current are less vulnerable because the difference between the upper and lower bounds (37 mV) is relatively small compared to 117 and 154 mV, the most likely range of driving forces across the Na channel.

The data in Table 2 (column 6) show that the coupling ratio is greater than 1 and less than 2.5. Previous electrophysiological experiments indicated that the electrogenic part of the sodium current was approximately 3.5 μA or 130 (nM/hr) (Miller et al., 1978). Assuming a midrange value for $I_{Pump}^K$, the total Na current through the pump is $130(\text{nM/hr}) + I_{Pump}^K(80\,\text{nM/hr}) \simeq 210(\text{nM/hr})$. This estimate of Na pump current is larger than the limit set by the flux data (column 6), and it leads to a coupling ratio of $\simeq 2.6$. All of the data, flux and electrophysiological, could be made self-consistent by assuming that $T_{Na} \simeq 0.1$ instead of 0.05 (legend to Table 2); experimentally, these values are difficult to distinguish (Miller & Steinberg, 1977*b*). This assumption does not alter the coupling ratio, nor is the $[K]_o$-induced change in Na pump current appreciably altered. In either case the data in Table 2 suggest that the coupling ratio does not increase with $[K]_o$.

[4] This conclusion was arrived at by comparing the upper (lower) bounds of the pump currents at 2 and 5 mM (Table 2, column 5). It might be argued that if one compared the upper bound at 2 mM with the lower bound at 5 mM, a very different conclusion could emerge for the changes in Na *and* K pump currents. Such a comparison, however, would be inappropriate since the assumed baseline potential, $V_m = -84$ mV (cell negative), was obtained from a very large sample of cells and tissues (*see* legend, Table 2) and because increasing $[K]_o$ from 2 to 5 mM always decreased the TEP and depolarized the apical membrane potential by approximately the same amount.

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