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Potassium Transport and Intracellular Potassium Activities in Rabbit Gallbladder

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Summary. Intracellular K activities, $(K)_c$, in rabbit gallbladder were determined using conventional and ion-selective microelectrodes. $(K)_c$ averaged 73 mM and was 1.5 times that predicted for an equilibrium distribution of the ion across both apical and basolateral membranes. Thus, K must be actively transported into the cell, and the responsible mechanism is almost certainly the Na–K exchange pump in the basolateral membrane.

Measurements of the bidirectional transepithelial fluxes of ^{42}K indicate that K is secreted into the mucosal solution at a rate of 0.8 μeq/cm^2 hr; this value is only 6% of the rate of transcellular Na absorption by this epithelium.

Calculation of the conductance of the basolateral membrane, G^s, reveals that it is too low to account for the maintenance of the steady-state $(K)_c$ by a 3 Na:2 K pump mechanism at the basolateral membrane if K exit across that barrier is entirely electrodiffusional.

Our results together with those of others strongly suggest that a significant fraction of "downhill" K exit from the cell across the basolateral membrane is nonconductive and coupled to the movement of some other ion, perhaps Cl.

Keywords rabbit gallbladder · potassium transport · intracellular potassium activity · membrane potentials

Introduction

Previous studies (Cremaschi, Hénin & Ferroni, 1974; Cremaschi & Hénin, 1975; Frizzell, Dugas & Schultz, 1975; Hénin & Cremaschi, 1975; Hénin, Cremaschi, Meyer & Brivio, 1976; Duffey, Turnheim, Frizzell & Schultz, 1978) have provided compelling evidence that transepithelial NaCl transport by rabbit gallbladder involves an electrically neutral influx mechanism(s) at the apical membrane which couples the uphill entry of Cl to the movement of Na down a favorable electrochemical gradient. This overall process requires no direct coupling to metabolic energy but, instead, is energized by the electrochemical potential difference for Na across the apical membrane which is established and maintained by the active extrusion of Na from the cell across the basolateral membrane. [1]

Our understanding of the mechanism responsible for the exit of Na from the cell is incomplete. It has been postulated on the basis of somewhat indirect evidence (Dietschy, 1964; Kaye, Wheeler, Whitlock & Lane, 1966; Frederiksen & Leyssac, 1969; van Os & Slegers, 1971) that, as in other epithelia, active Na extrusion in rabbit gallbladder is coupled to active K uptake. However, direct evidence bearing on the relation between transcellular Na transport and K movements across the basolateral membrane is lacking.

Likewise, the mechanism(s) responsible for Cl exit from the cell remain(s) to be elucidated. The electrochemical potential difference for Cl across the basolateral membrane, measured by Duffey et al. (1978), favors the diffusion of Cl across that barrier. However, evidence from several laboratories (Cremaschi & Hénin, 1975; Hénin & Cremaschi, 1975; van Os & Slegers, 1975) indicates that the Cl conductance of the basolateral membrane is too low to permit Cl exit to be entirely electrodiffusional. Thus, it appears that Cl exit must be largely nonconductive and coupled to the countertransport of an anion, for example, HCO_3, the co-transport of a cation, possibly K, or both.

[1] Neutral NaCl influx may result from a NaCl co-transport mechanism as in the model suggested by Frizzell et al. (1975) or, alternatively, by linked Na–H and Cl–HCO_3 countertransport processes operating in parallel. The latter mechanism was initially suggested for mammalian small intestine by Turnberg, Bieberdorf, Morawski and Fordtran (1970) and is supported by results obtained using brush-border vesicles from small intestine (Liedtke & Hopfer, 1977; Mürer, Hopfer & Kinne, 1976). It should be clear, however, that if CO_2 and H_2O are distributed at equilibrium across the apical membrane the energy for these countertransport processes must ultimately be derived from the Na pump and the Na-gradient.

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The purpose of this study was to examine potassium transport by rabbit gallbladder and intracellular potassium activities in an effort to gain further insight into the transport mechanisms operating at the basolateral membrane.

Materials and Methods

Tissues and Solutions

New Zealand white rabbits (2–4 kg) were sacrificed by intravenous injection of pentobarbital, and the gallbladder was removed and opened as described previously (Frizzell et al., 1975). For transepithelial K flux determinations, the tissue was mounted in chambers intact, whereas for measurement of the intracellular electrical potential and K activity, gallbladders were stripped of their underlying musculature (Frizzell et al., 1975) before mounting.

The composition of the Ringer's solution was (mM): 140 Na, 124 Cl, 21 HCO_3, 5.4 K, 2.4 HPO_4, 0.6 H_2PO_4, 1.2 Mg, 1.2 Ca, and 10 glucose. The pH of the solution when equilibrated with 95% O_2/5% CO_2 was 7.4. The experiments were conducted at 37 °C.

Transepithelial Potassium Fluxes

The transepithelial unidirectional fluxes of K from mucosa to serosa, J_K^{ms}, and from serosa to mucosa, J_K^{sm}, were determined using hemi-bladders from the same animals. The tissues were mounted in Ussing chambers exposing 0.64 cm^2 of surface area, and both surfaces were bathed by 10 ml oxygenated Ringer's solutions. Generally, 40 min were required for the transepithelial potential, ψ^{ms}, and resistance, R_t, to become stable. ^{42}K (New England Nuclear) was then added to the appropriate half-chamber, and after 40 min J_K^{ms} and J_K^{sm} were determined as described previously (Schultz & Zalusky, 1964) from at least three 20-min flux periods. Since ψ^{ms} is near zero in rabbit gallbladder, the fluxes were determined under open-circuit conditions. The samples were analyzed for ^{42}K using a liquid scintillation counter (Tracor Analytic) and corrected for decay.

Intracellular Electrical Potentials and Potassium Activities

Conventional microelectrodes were fabricated from thin-walled fiber-filled glass capillaries having an outer diameter of 1.2 mm and an inner diameter of 0.68 mm (WP Instruments). After cleaning with nitric acid followed by rinsing with deionized H_2O, the capillaries were pulled to a fine tip (diameter <0.2 μm) using a horizontal puller (Sutter Instrument Company, Model P-77 Brown-Flaming) and back-filled with 0.5 M KCl using a fine-gauge needle. Contact between the salt solution and the electrical circuit was made with an Ag/AgCl electrode. These microelectrodes had a tip resistance of approximately 100 MΩ when the tip was immersed in 0.5 M KCl. As discussed by Fromm and Schultz (1981), the use of 0.5 M KCl rather than 3 M KCl reduces K-leakage without significantly affecting tip potentials.

Microelectrodes, pulled exactly as described above, were used to make K-selective microelectrodes. As described previously by Palmer and Civan (1975, 1977), the barrel of the electrode was rendered hydrophobic by exposure to vapors of dimethyldichloro- or methyltrichlorosilane for 2 min. The electrodes were then cured in an oven for 1 hr at 120 °C. After cooling, approximately 0.02 ml of Corning # 477317 liquid-ion-exchanger (Dow Corning Corporation) was injected into the silanized untapered end with a fine needle. The tapered shank of the electrode was allowed to fill with exchanger by capillary action. At this point, the microelectrode could be stored for up to 2 days. Prior to the experiment, excess exchanger was removed from the untapered end and the barrel was back-filled with 0.5 M KCl. Contact between the salt solution and the electrical measuring system was made with an Ag/AgCl electrode.

K-selective microelectrodes were calibrated before and after each experiment in various concentrations of KCl and in the standard Ringer's solution. The K activity of the solutions was calculated as described by Fujimoto and Kubota (1976). The slopes of the lines relating ln (K) and the electrical potential recorded by the microelectrodes (E_t) averaged 26.2 ± 0.2 mV in reasonable agreement with the value of 25.2 mV predicted for an ideal electrode. The selectivity constant of the electrode for Na calculated from the difference in the output of the K microelectrode (E_t) in a pure KCl solution and a Ringer's solution having the same K activity was 0.018 ± 0.002; this value is in good agreement with those reported by others (e.g., Lev & Armstrong, 1975; Fujimoto & Kubota, 1976; Reuss & Weinman, 1979). These electrodes had tip resistances between 10^9–10^{10} Ω.

For measurements of intracellular electrical potentials and K activity, "mucosal strips" were mounted mucosal surface up in a chamber exposing 0.13 cm^2 of the tissue and both surfaces of the tissue were superfused with oxygenated Ringer's solution. The transepithelial potential difference with respect to the mucosal solution, ψ^{ms}, was monitored by an automatic voltage clamp in contact with the bathing solutions via calomel cells and Ringer-agar bridges. Since the spontaneous ψ^{ms} is nearly zero, the tissue was not short circuited. Constant-current pulses generated by the voltage clamp were passed across the epithelia via Ag/AgCl electrodes in each bathing solution.

The potential difference across the apical membrane with respect to the mucosal solution, ψ^{mc}, was measured with conventional microeletrodes advanced into the cell using a hydraulic microdrive (Narishige, Ltd., Model MO-8). The output of this electrometer with respect to a calomel cell in contact with the mucosal solution was monitored by another electrometer (WP Instruments, 750). This arrangement assured that current passed across the epithelium would not flow through the potential measuring system.

The ratio of the mucosal to the serosal membrane resistances, (R^m/R^s), was calculated from the deflections of ψ^{mc} and ψ^{ms} produced by brief bipolar transepithelial current pulses having a magnitude of 385 μA/cm^2, a duration of 2 sec and a period of 10 sec.

The change in the output of K-selective microelectrodes when advanced into the cell (ΔE_t) was monitored as described above for conventional microelectrodes. ψ^{ms} and ψ^{mc} or ΔE_t were recorded on a Gould chart recorder (Model 2400).

The intracellular K activity, $(K)_c$, was calculated from ΔE_t and ψ^{mc} using the relation:

$$\Delta E_t = S \ln \{(K)_c/[(K)_m + k_{KNa}(Na)_m]\} + \psi^{mc} \quad (1)$$

where S is the slope of the relation between E_t and ln (K) determined from the calibrations; k_{KNa} is the selectivity constant for Na over K; and the subscripts c and m designate the intracellular and mucosal compartments, respectively (Lev & Armstrong, 1975).[2]

All results are expressed as the mean ± the standard error of mean. When required, the significance of the difference between two means was determined by the paired or unpaired Student

[2] The intracellular Na activity is likely to be less than 20 mM (Reuss & Weinman, 1979; Graf & Giebisch, 1979). Thus, the contribution of cell Na to the intracellular potential sensed by the K-selective electrode is negligible.

t-test and a value of $P<0.05$ was accepted as the level of significance.

Results

Transepithelial Potassium Fluxes

Transepithelial unidirectional fluxes of K were determined as described above on paired hemi-bladders from six rabbits. J_K^{ms} averaged 0.92 ± 0.13 μeq/cm^{2}hr and J_K^{sm} averaged 1.74 ± 0.37 μeq/cm^{2}hr; these values differ significantly ($P<0.01$) and indicate that K is secreted by this preparation at a rate of 0.82 ± 0.19 μeq/cm^{2}hr. Inasmuch as ψ^{ms} is close to zero (and, if anything, is slightly negative), this secretion must be "active" and proceed via the transcellular route.

Previous studies of K transport by rabbit gallbladder did not detect net secretion *in vivo* (Dietschy & Moore, 1964) or *in vitro* (Wheeler, 1963; Dietschy and Moore, 1964). However, it is of interest that the flux ratio, (J_K^{ms}/J_K^{sm}), observed by Wheeler (1963) was consistently less than that predicted by the Ussing flux ratio equation and is, therefore, consistent with a slow rate of K secretion that could have gone undetected when examining net fluxes. Reuss (1981) has provided indirect but compelling evidence for K secretion by *Necturus* gallbladder *in vitro*.

Intracellular Electrical Potentials and Potassium Activities

Typical recordings using both conventional and K-selective microelectrodes are shown in Fig. 1. Successful punctures with conventional microelectrodes exhibited an abrupt negative deflection which was stable for at least 30 sec with an abrupt return to baseline after retracting the microelectrode from the cell. (R^m/R^s) for such a puncture was always $\geqq 1.0$. In contrast, punctures with K-selective microelectrodes typically displayed little or no change in the output of the K-selective microelectrode ($\Delta E_t \simeq 0$) although values of $(R^m/R^s) \geqq 1.0$ indicated successful impalements. Less frequently, as illustrated in Fig. 2, ΔE_t was slightly negative or slightly positive. In either instance, in every tissue (R^m/R^s) determined with conventional KCl-filled microelectrodes was in good agreement with that obtained using K-selective microelectrodes.

Histograms of all successful punctures, shown in Fig. 3, demonstrate that both ψ^{mc} and ΔE_t appear to be distributed about a single maximum, indicating that the variations described above do not result from the impalement of distinctly different populations of cells.

The potential difference across the apical membrane, ψ^{mc}, averaged -64 ± 2 mV ($n=9,37$)[3] in reasonable agreement with values reported previously by this (Frizzell et al., 1975; Duffey et al., 1978) and other laboratories (Cremaschi et al. 1974; van Os & Slegers, 1975; Hénin et al., 1976, 1977). (R^m/R^s) averaged 2.5 ± 0.02; this value is higher than the value

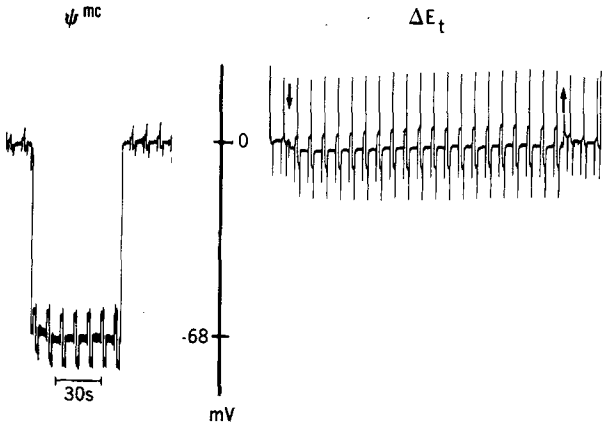

Fig. 1. Successful impalements using conventional (ψ^{mc}) and K-selective (ΔE_t) microelectrodes in the same tissue. Advancement of the ion-selective electrode into the cell and its retraction are indicated by the arrows

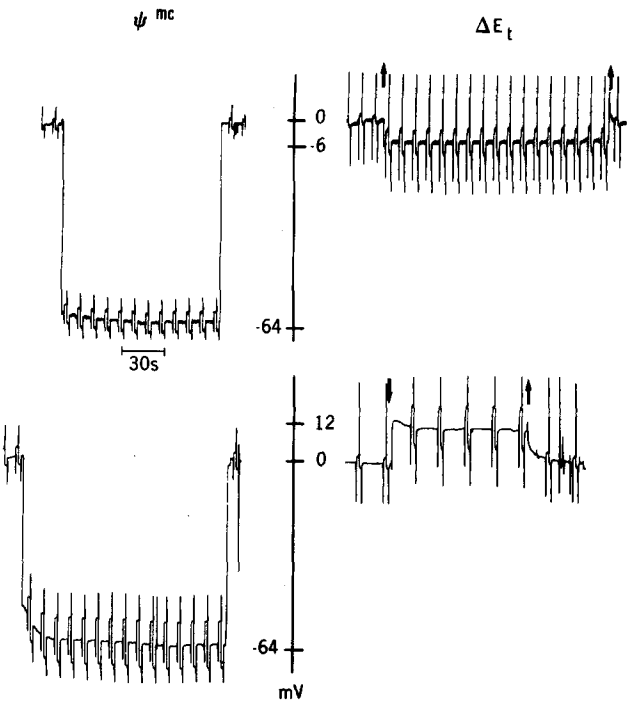

Fig. 2. Successful impalements with conventional and K-selective microelectrodes in which ΔE_t is either negative (upper trace) or positive (lower trace)

[3] $n=x,y$ indicates that the data are the average of y successful punctures on x tissues.

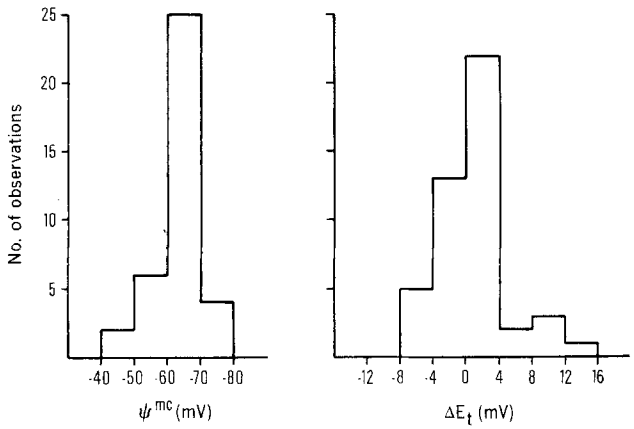

Fig. 3. Histograms of all successful impalements with conventional (ψ^{mc}) and K-selective (ΔE_t) microelectrodes

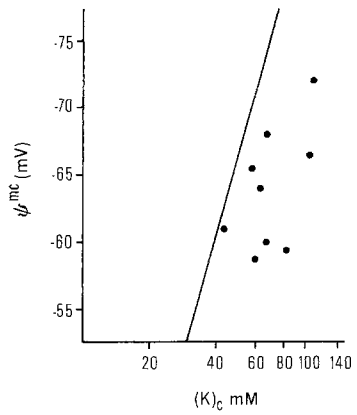

Fig. 4. Relation between the intracellular K activity, $(K)_c$, and ψ^{mc} determined from impalements with conventional and K-selective microelectrodes. Each point represents the $(K)_c$ (calculated from the mean ψ^{mc} and ΔE_t) and the average ψ^{mc} determined in each tissue. The solid line indicates the relation expected for an equilibrium distribution of the ion

of 1.09 reported previously for this tissue by Henin et al. (1977), but is in good agreement with that reported more recently for *Necturus* gallbladder (Reuss, 1979).

ΔE_t averaged 0.9 ± 2 mV ($n=9,45$) and was not significantly different from zero. (R^m/R^s) for these punctures averaged 2.2 ± 0.2 and did not differ from that observed for impalements with conventional microelectrodes.

The intracellular K activity, $(K)_c$, calculated using Eq. (1) averaged 73 ± 7 mM in good agreement with values determined using similar techniques in other epithelia (Lee & Armstrong, 1972; Lewis, Wills & Eaton, 1978; Delong & Civan, 1980; Wills, Lewis & Eaton, 1979; Reuss & Weinman, 1979; Fujimoto, Kazuyot & Kubota, 1980). As illustrated in Fig. 4, in every tissue $(K)_c$ was greater than the activity predicted by the Nernst equation for an equilibrium distribution of a monovalent cation, $(K)_c^e$; the average $(K)_c$ is 1.5 times the average $(K)_c^e$ of 43 mM. Since $\psi^{ms} \simeq 0$, the electrochemical potential differences for K in mV, $(\Delta \tilde{\mu}_K/\mathcal{F})$, across the apical and basolateral membranes are equal and averaged 12 mV.[4]

Discussion

Intracellular Potassium Activity and Potassium Secretion

The determination of the intracellular K activity in these studies is complicated by two factors. First, ideally, intracellular activities should be determined using double-barreled microelectrodes that permit simultaneous measurements of ψ^{mc} and ΔE_t in the same cell. However, as discussed previously (Duffey et al., 1978): (i) rabbit gallbladder is comprised of a single layer of histologically very similar cells (Blom & Helander, 1977); and (ii) we and other investigators have noted remarkably little scatter in measured values of ψ^{mc} within a given tissue and among different tissues. Thus, the use of separately determined values of ψ^{mc} and ΔE_t to calculate $(K)_c$ seems to be justifiable and perhaps "safer" than the use of double-barreled microelectrodes which may produce serious impalement artifacts when employed to impale small animal cells.

Second, among the usual criteria for a "successful impalement" is the observation of an abrupt deflection which reaches a stable value. This criterion was easily satisfied in these studies using conventional KCl-filled microelectrodes for measurement of ψ^{mc}. However, this criterion cannot be applied to the use of ion-selective microelectrodes if the intracellular ion in question is distributed at or close to electrochemical equilibrium inasmuch as there will either be no deflection upon impalement of the cell or the deflection will be unimpressively small. In these circumstances, one criterion that can be employed for judging a successful impalement is evidence that the microelectrode has abruptly penetrated a resistive barrier and that the value of (R^m/R^s) determined experimentally from the value of ($\Delta\psi^{mc}/\Delta\psi^{ms}$) (in response to external current pulses) does not differ significantly from that determined using conventional KCl-filled microelec-

[4] It should be noted that the fact that $(\Delta \tilde{\mu}_K \mathcal{F}) = 12$ mV whereas ΔE_t did not differ significantly from zero is due to the "nonideal" behavior of the ion-selective microelectrode, particularly the significant contribution to the electrode potential resulting from Na interference when the tip is in the mucosal solution. Thus, the accuracy of $(K)_c$ calculated using Eq. (1) is dependent on, among other things, the accuracy of k_{KNa}. An alternate approach (suggested by one of the reviewers) that circumvents the need to rely on k_{KNa} is to subtract ψ^{mc} from the intracellular potential recorded by the K-electrode and then determine $(K)_c$ directly from the calibration line. A recalculation of a set of randomly selected data using this method yielded results that were essentially identical with those obtained using Eq. (1)).

trodes. This criterion was employed in the present studies. Additional criteria have been suggested by Reuss and Weinman (1979).

Given these reservations, our data indicate that rabbit gallbladder epithelial cells actively accumulate K against an electrochemical potential difference of approximately 12 mV. The value of $(K)_c = 73 \pm 7$ mM is in reasonably good agreement with the values reported for several other epithelia including *Necturus* gallbladder (Reuss & Weinman, 1979); bullfrog small intestine (Lee & Armstrong, 1972); rabbit descending colon (Wills et al., 1979; unpublished results from this laboratory); bullfrog (Fujimoto et al., 1980) and *Necturus* proximal tubule (Kimura & Spring, 1979); and rabbit (Lewis et al., 1978) and toad urinary bladder (Delong & Civan, 1980). Finally, assuming that intracellular K is distributed in free-solution with an activity coefficient of 0.75, the intracellular concentration calculated from these data is 97 mM; this value is in reasonable agreement with the total cell K concentration of 85 ± 7 mM determined chemically by Frizzell et al. (1975).

On the basis of these findings and the findings that: (i) Na–K activated ATPase has been localized to the basolateral membranes of rabbit gallbladder (Kaye et al., 1966); (ii) the activity of this enzyme parallels the rate of NaCl absorption (van Os & Slegers, 1971); and, (iii) transport is inhibited by removing K from the serosal solution (Frederiksen & Layssac, 1969) and by the presence of ouabain in the serosal solution (Dietschy, 1964; Martin & Diamond, 1966), it seems reasonable to conclude that the mechanism responsible for active K accumulation by the cell is a Na–K ATPase located at the basolateral membrane and that this mechanism is also responsible for active transcellular Na transport.

These findings, together with the electrophysiological evidence that the mucosal membrane is predominantly permeable to K (Cremaschi et al., 1974), provides a ready explanation for the K secretion observed in this study; indeed, the finding of K secretion was entirely predictable.

Ion Movements Across the Apical and Basolateral Membranes

These studies were undertaken in order to gain further insight into the mechanism(s) responsible for the movements of Na, K and Cl across the basolateral membrane of rabbit gallbladder cells. The results, however, together with the results of previous studies on this preparation raise more perplexing questions than they resolve.

The transepithelial conductance of rabbit gallbladder in these studies averaged 36 ± 3 mS/cm^2, a value in excellent agreement with that reported previously by several groups (Wright, Barry & Diamond, 1971; Frizzell et al., 1975). The conductance of the shunt pathway accounts for approximately 95% of the total conductance (Wright et al., 1971; Frizzell et al., 1975; Hénin et al., 1977) so that the conductance of the transcellular pathway is 1.8 mS/cm^2. Since (R^m/R^s) averaged 2.4, it follows that the apparent resistance of the apical membrane is 390 Ωcm^2 and that of the basolateral membrane is 160 Ωcm^2; the latter value is very similar to that reported by Hénin et al. (1977).

We are now in a position to estimate the diffusional flows of K out of the cell across the apical and basolateral membranes driven by the electrochemical potential difference for K across these barriers, 12 mV, from the relation:

$$J_K = G_K \, (\Delta\tilde{\mu}_K/\mathcal{F}^2) \qquad (2)$$

where G_K is the partial ionic conductance of the barrier to K.

If, as suggested by the data of Cremaschi et al. (1974) and Hénin and Cremaschi (1975), the apical membrane is highly K-selective, $G_K^m \simeq (1/390) \simeq 2.56$ mS/cm^2 and, from Eq. (2), $J_K^m = 1.2$ μeq/cm^{2}hr; this value is in reasonable agreement with the rate of net K secretion actually observed in these studies (0.82 ± 0.19 μeq/cm^{2}hr).

If the basolateral membrane is also predominantly permeable to K (Cremaschi et al., 1974; Hénin & Cremaschi, 1975; van Os & Slegers, 1975), $G_K^s \simeq (1/160) \simeq 6.25$ mS/cm^2 and, from Eq. (2), $J_K^s = 2.8$ μeq/cm^{2}hr.

It follows that the minimum rate of K uptake by the basolateral pump must be $J_K^m + J_K^s = 4.0$ μeq/cm^{2}hr. Although we did not measure the rate of transepithelial Na transport in these studies, the value of 14 μeq/cm^{2}hr reported by Frizzell et al. (1975) was determined under essentially identical conditions; this value is also in excellent agreement with the rate determined chemically by Wheeler (1963) and that estimated from the rate of volume absorption (Machen & Diamond, 1969; Cremaschi & Hénin, 1975). Assuming this to be the case in the present studies, the stoichiometry of the Na:K pump would be (14/4.0) or approximately (3.5:1). Although certainly possible, this value differs significantly from the 3 Na:2 K stoichiometry estimated for some nonepithelial cells (Glynn & Karlish, 1975) as well as some epithelial cells (Neilson, 1979; Lewis, 1981).

Further, by assigning the *entire* basolateral conductance to K, we accentuate the problem of accounting for Cl exit across the basolateral membrane down its electrochemical potential difference!

If, on the other hand, the pump stoichiometry is 3 Na:2 K, then K uptake into the cell via this

mechanism would be more than twice the rate at which it can exit by simple electrodiffusion. Under these circumstances, approximately 5 μeq/cm²hr would have to exit via a nonconductive mechanism which raises the possibility of a neutral (nonconductive) KCl exit process driven by the combined electrochemical differences for K (12 mV) and Cl (23 mV) (Duffey et al., 1978).[5] However, it is obvious that such a mechanism cannot account for the entire rate of Cl exit (14 μeq/cm²hr) unless the rate of K uptake by the Na:K exchange pump exceeds the rate of Na extrusion; to our knowledge, this has not been reported for any system.

Clearly, other possibilities can be conjectured, but further speculation on this point does not seem warranted. Suffice it to say that the movements of Na, K and Cl across the basolateral membrane of rabbit gallbladder cannot be accounted for quantitatively at this time by a simple model; and the stimulatory role of HCO_3 in this system (Diamond, 1964; Martin, 1974; M.C. Dugas, R.A. Frizzell and S.G. Schultz, *unpublished observations*) has yet to be adequately elucidated.[6] Identical problems seem to confront three other epithelia that possess NaCl co-transport mechanisms; namely, *Necturus* gallbladder (Reuss, Weinman & Grady, 1980), *Necturus* renal proximal tubule (Shindo & Spring, 1981) and flounder small intestine (R. Frizzell, *personal communication*).

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[5] Reuss et al. (1980) have recently entertained a similar possibility for *Necturus* gallbladder.

[6] Duffey et al. (1978) raised the possibility of a Cl–HCO_3 exchange across the basolateral membrane. The present results raise the possibility of a K–H exchange in parallel with a Cl–HCO_3 exchange. Na–H, K–H, and Cl–HCO_3 countertransport processes have been implicated in the volume regulatory responses of *Amphiuma* erythrocytes (Cala, 1980).

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