Sodium Chloride Transport by Rabbit Gallbladder

Direct Evidence for a
Coupled NaCl Influx Process

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ABSTRACT The results of the present study indicate that NaCl transport by in vitro rabbit gallbladder must be a consequence of a neutral coupled carrier-mediated mechanism that ultimately results in the active absorption of both ions; pure electrical coupling between the movements of Na and Cl can be excluded on the grounds of electrophysiologic considerations. Studies on the unidirectional influxes of Na and Cl have localized the site of this coupled mechanism to the mucosal membranes. Studies on the intracellular ion concentrations and the intracellular electrical potential are consistent with the notion that (a) the coupled NaCl influx process results in the movement of Cl from the mucosal solution into the cell against an apparent electrochemical potential difference; (b) the energy for the uphill movement of Cl which is maintained by an active Na extrusion mechanism located at the basolateral membranes; and (c) Cl exit from the cell across the basolateral membranes is directed down an electrochemical potential gradient and may be diffusional. Finally, as for the case of rabbit ileum, the coupled NaCl influx process is inhibited by elevated intracellular levels of cyclic 3',5'-adenosine monophosphate. A working model for transcellular and paracellular NaCl transport by in vitro rabbit gallbladder is proposed.

INTRODUCTION
The results of a number of in vitro studies have suggested that a neutral, coupled carrier mechanism is responsible for Na and Cl absorption by fish (1) and rabbit gallbladder (2–4). Support for this notion rests mainly on the observations that absorption of Na and Cl is not associated with a significant spontaneous transepithelial electrical potential difference (PD) when the mucosal and serosal solutions have identical compositions. In addition, replacement of either Na or Cl with nontransported ions abolishes net ion and fluid absorption without affecting the transepithelial PD. Thus, coupling
between the net fluxes of Na and Cl does not seem to be "electrical," as is the case for a number of epithelia, but appears to result from a direct one-for-one interaction between these ions and a cellular transport mechanism(s). However, the site of this inferred interaction between Na and Cl transport has not been localized to either the mucosal or serosal limiting membranes of the epithelial cell, although the possibility of a neutral NaCl pump located at the serosal membranes has been suggested (5). Further, because rabbit and fish gallbladder are characterized by very low transepithelial resistances, the possibility has been raised that coupling between Na and Cl absorption is electrical but that the PD (secondary to active Na absorption) is markedly attenuated by highly conductive cell membranes or extracellular pathways (6–8).

Recent studies of Na and Cl absorption by rabbit ileum have identified a neutral, coupled NaCl transport mechanism at the brush border which plays a major role in active Na and Cl absorption (9, 10). If either Na or Cl in the mucosal solution are replaced with nontransported ions such as choline or sulfate, influx of both ions via this process is abolished. The similarity between coupled NaCl influx across the brush border of rabbit ileum and NaCl absorption by rabbit gallbladder prompted an investigation of ion transport by the gallbladder epithelium. The results of these studies disclose the presence of a coupled, neutral NaCl influx process at the mucosal membrane which is ultimately responsible for active transepithelial transport of Na and Cl; purely electrical coupling between the movements of these two ions can be excluded. In addition, our results suggest that the driving force for active Cl absorption may be derived from the electrochemical gradient for Na across the mucosal membrane via this cotransport process. Finally, evidence is presented that as for the case in rabbit ileum (9), the neutral NaCl influx process in rabbit gallbladder is inhibited by elevated levels of intracellular 3′,5′-cyclic adenosine monophosphate (cAMP).

METHODS

Male white rabbits (~3 kg) were sacrificed with pentobarbital and the gallbladder was excised by carefully severing its membranous attachments to the liver. The sac was then opened along its hepatic border, yielding a flat sheet which was rapidly washed free of bile and transferred to a buffer-filled petri dish in order to minimize prolonged contact of the tissue with bile salts. The normal electrolyte solution used in these studies contained: NaCl, 140; KHCO₃, 10; K₂HPO₄, 1.2; KH₂PO₄, 0.2; CaCl₂, 1.2; and MgCl₂, 1.2. This solution had a pH of 7.2 when gassed with 95% O₂-5% CO₂ at 37°C. Na-free or Cl-free solutions were prepared by isotonic replacement of NaCl with choline chloride or with Na₂SO₄ and mannitol; chloride-free solutions contained the sulfate salts of Mg and Ca in place of their Cl salts.

Transmural unidirectional fluxes of ²²Na, ³⁶Cl, or ⁸⁶SO₄ and the transepithelial PD and tissue resistance were determined using the techniques described by Schultz.
and Zalusky (11). The half-chambers had an exposed serosal surface area of 0.33 cm². No attempt was made to short-circuit the epithelium during flux determinations because the spontaneous transmural PD's were always less than 1.0 mV, usually serosa negative with respect to mucosa; as will be discussed below, this PD could not significantly affect diffusional fluxes across the tissue. A period of 30 min was allowed to elapse after the addition of the isotopes to ensure the achievement of a steady state. Mucosa-to-serosa fluxes ($J_{m,s}$) were then determined for a 40-min period. The chambers were then drained and refilled and the serosa-to-mucosa flux ($J_{s,m}$) of the same ion was determined during a second 40-min period using the same tissue segment. During each flux period, samples were taken at 5-min intervals and the mean of these fluxes represents the unidirectional flux for that tissue. Preliminary experiments indicated that this approach permits reliable determinations of $J_{m,s}$ and $J_{s,m}$ on the same tissue since steady-state unidirectional fluxes are observed for at least 1.5 h. Since two segments of tissue were obtained from one gallbladder, each of which was mounted in an identical apparatus, bidirectional transmural fluxes of either Na or Cl were determined under control and experimental conditions using tissue from the same animal.

Unidirectional influxes of Na and Cl from the mucosal solution across the mucosal membrane into the epithelium ($J_{m,m}$) were measured using a modification of the method described by Schultz et al. (12). Tissues were mounted, mucosal surface up, in Lucite chambers which permitted exposure of the mucosal surface alone to solutions of desired compositions; the serosal surface (0.155 cm²) rested on moistened filter paper. After a 20-min exposure to a nonradioactive solution, the mucosal surface was exposed to a test solution having an identical composition but also containing tracer amounts of $[^3H]$inulin and either $^{22}Na$ or $^{36}Cl$. After a brief exposure, this solution was withdrawn and the exposed area was rinsed briefly with an ice-cold isotonic mannitol solution. The tissue was then punched out and extracted in 0.1 N HNO₃ for at least 2 h. Tissue extract and aliquots of the mucosal solution were assayed for $[^3H]$inulin and either $^{22}Na$ or $^{36}Cl$ simultaneously, and uptake of $^{22}Na$ or $^{36}Cl$ by the tissue across the mucosal surface was calculated after correction for the volume of adherent radioactive solution given by the inulin space. The applicability of this technique to the measurement of unidirectional Na and Cl influxes in rabbit gallbladder is illustrated by the data in Fig. 1. The uptakes of Na and Cl from the mucosal solution are linear functions of time of exposure of the mucosal surface to the test solution for at least 45 s. Analysis of the serosal filter paper showed that neither Na nor Cl crossed the tissue from the mucosal solution during this brief exposure. Thus, in all experiments, the mucosal surface was exposed to the test media for 45 s. In most instances, four influxes could be determined on tissue from a single gallbladder.

The intracellular electrical potential with reference to the mucosal solution ($\psi_{m,s}$) was measured using the techniques described by Rose and Schultz (13) for rabbit ileum. Microelectrodes were prepared from 1.5 mm OD borosilicate glass tubing and filled with 3 M KCl. Electrodes having a tip resistance of 5–10 MΩ and tip potentials of less than 5 mV were connected via a calomel half-cell to a Keithley model 600B electrometer (Keithley Instruments, Inc., Cleveland, Ohio). In these experiments, the serosal muscle layers were stripped from the epithelium using glass microscope slides (henceforth, this preparation will be referred to as “mucosal strips”) because spontaneous muscle contraction made prolonged micropuncture of whole-thickness
gallbladders difficult. The mucosal strip was mounted mucosal surface up between the halves of a Lucite chamber having an aperture of 1.13 cm². The mucosal surface of the tissue was impaled manually using a Brinkmann micromanipulator (Brinkmann Instruments, Inc., Westbury, N. Y.) or automatically using a David Kopf (model 607) hydraulic microdrive (David Kopf Instruments, Tujunga, Calif.) set at a minimum advance of 1 μm. Potential differences with respect to the mucosal solution were recorded using a Texas Instruments dual-channel recorder (Texas Instruments Inc., Houston, Texas). Criteria for successful impalements were those detailed by Rose and Schultz (13) for rabbit ileum and were not difficult to meet using the techniques described (Fig. 2).

Intracellular Na and K concentrations were determined on mucosal strips using the methods described by Schultz et al. (14) and intracellular Cl concentrations were determined using NaCl as described by Frizzell et al. (15). Tissue from half of one bladder was used to determine intracellular Na and K concentrations ([Na]ᵢ and [K]ᵢ) and the other half was employed for the [Cl]ᵢ determinations. [³⁶Cl]inulin served as the extracellular space marker in all of these experiments. Preliminary determinations of inulin and Cl spaces per milligram dry weight as a function of time indicated that a 30-min incubation was sufficient to achieve steady-state distributions of these solutes with their respective spaces. After the incubation, wet and dry weights of each tissue segment were determined and the dried tissues were extracted for 48 h at 4°C. For those half-bladders in which [Na]ᵢ and [K]ᵢ were determined, the extraction medium was 15 mM LiCl solution and Na and K concentrations of the tissue extracts and incubation media were determined using an internal standard Instrumentation Laboratory model 143 Flame Photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). For determination of [Cl]ᵢ, half-bladders were extracted using 0.1 N HNO₃, and aliquots of incubation media and tissue extracts were analyzed for ³⁶Cl and [¹⁴C]inulin using liquid scintillation spectrometry.

All studies were performed at 37°C. Intracellular ion concentrations are expressed as millimoles per liter intracellular water, fluxes as μmol/cm² h, and all errors are determined using ³⁶Cl as described by Frizzell et al. (15).
RESULTS AND DISCUSSION

Transepithelial Electrical Parameters

When both surfaces of the tissue were bathed with the normal electrolyte solution, the spontaneous transepithelial electrical potential difference with respect to the mucosal solution averaged $-0.8 \pm 0.1$ mV ($n = 18$). When the tissue was bathed with Na-free choline media or Cl-free sulfate media, the PD tended to be closer to zero but because of the low values observed under control conditions and inevitable small electrode asymmetries these differences are not significant. These observations are in excellent agreement with those reported previously (2-4, 16). The transepithelial resistance determined by passing a brief (10-15 s) 100-μA pulse of direct current across the tissue and correcting for fluid resistance averaged $26.3 \pm 1.1$ Ω cm$^2$ ($n = 18$) when the tissue was bathed with the normal electrolyte solution; in general, the resistance was lowest immediately after mounting the tissue and increased slowly during the ensuing 2-3 h. It should be stressed that determinations of the tissue resistance using these techniques is subject to considerable error because the correction for fluid resistance is more than 30% of the total resistance. Nevertheless, the average value in these studies is in excellent agreement with that reported by Wright et al. (17).

Transmural Na and Cl Fluxes

Unidirectional transepithelial fluxes of Na and Cl across rabbit gallbladder are given in Table I. Control fluxes were determined using the normal electrolyte media and the effects of ion replacements on these fluxes were determined on tissue from the same animal as described above. The measured unidirectional Na and Cl fluxes were large, reflecting the high ionic conductance of this tissue (30-40 mmho/cm$^2$). In media containing 140 mM Na and

<table>
<thead>
<tr>
<th></th>
<th>$J_{Na}^{m}$</th>
<th>$J_{Na}^{s}$</th>
<th>$J_{Na}^{net}$</th>
<th>$J_{Cl}^{m}$</th>
<th>$J_{Cl}^{s}$</th>
<th>$J_{Cl}^{net}$</th>
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<tr>
<td>Normal buffer</td>
<td>35.1±2.4</td>
<td>22.1±0.6</td>
<td>12.9±2.1</td>
<td>28.1±2.3</td>
<td>13.3±2.3</td>
<td>14.8±0.7</td>
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<tr>
<td>Cl-free buffer</td>
<td>22.1±1.1</td>
<td>21.3±1.1</td>
<td>0.7±0.3</td>
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<tr>
<td>Na-free buffer</td>
<td>---</td>
<td>---</td>
<td>12.7±1.8</td>
<td>12.3±2.0</td>
<td>0.4±0.4</td>
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</tr>
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</table>

$m$ and $s$ designate the mucosal and serosal solutions, respectively, and $J_{i}^{j}$ designates the unidirectional flux of ion $i$ from the $j$th compartment to the $k$th compartment; $J_{i}^{net} = J_{i}^{m} - J_{i}^{m}$. The fluxes are in micromoles per square centimeter hour and each value is the mean ± SEM of paired determinations on five gallbladders.
145 mM Cl, the net fluxes of these ions did not differ significantly and averaged approximately 14 μmol/cm² h. When the mucosal and serosal solutions were rendered Cl free, net Na absorption was essentially abolished due to a decrease in mucosa-to-serosa flux (Jₙₐ₉) alone with no change in the serosa-to-mucosa flux (Jₕₑ₉). Similarly, net Cl flux was not significantly different from zero when the mucosal and serosal solutions were Na free and, again, the abolition of J₉ₚCl was the result of a decrease in J₉ₑₚCl alone. In neither case was the serosa-to-mucosa flux of these ions affected by co-ion replacement; as will be discussed below, this finding has significant bearing on the mechanism of "coupling" between Na and Cl transport.

The effects of 7.5 mM cAMP on transmural Na and Cl fluxes are given in Table II. The cyclic nucleotide was added to the mucosal and serosal bathing solutions after a 40-min control period in which bidirectional transmural fluxes of either Na or Cl were measured on adjacent tissue segments. The large unidirectional fluxes in control tissues compared to those given in Table I probably reflect an increased passive permeability of these tissues inasmuch as (a) the net fluxes of Na and Cl did not differ significantly from each other or from the values shown in Table I, and (b) the value of JₙₑₚCl/JₙₑₚNa from the data in Table II (1.5) agrees favorably with the value of 1.7 obtained from the data given in Table I; as will be discussed below, JₚₑₚCl of both Na and Cl appear to be largely confined to the passive conductance or extracellular pathway(s). In the presence of cAMP, mucosa-to-serosa fluxes of both Na and Cl were significantly reduced with no significant change in the serosa-to-mucosa flux of these ions. These changes resulted in equivalent reductions of the net fluxes of both Na and Cl, although significant absorption remained. In several experiments in which higher concentrations of cAMP were employed, net NaCl

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>EFFECT OF cAMP ON TRANSMURAL SODIUM AND CHLORIDE FLUXES</th>
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<tbody>
<tr>
<td></td>
<td>Na fluxes</td>
</tr>
<tr>
<td></td>
<td>Control +7.5 mM cAMP</td>
</tr>
<tr>
<td>JₙₑₚNa</td>
<td>45.6±2.3</td>
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<tr>
<td>JₚₑₚNa</td>
<td>29.7±1.3</td>
</tr>
<tr>
<td>JₙₑₚCl</td>
<td>15.9±2.6</td>
</tr>
<tr>
<td>ΔJₙₑₚ</td>
<td>10.1±3.8</td>
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<tr>
<td></td>
<td>10.7±3.7</td>
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</tbody>
</table>

See legend to Table I. Each value represents the mean ± SEM of paired experiments on five gallbladders.

1 The data given in Tables I, III, and IV were obtained by one investigator (R.A.F.) and those reported in Table II were obtained by another (M.D.). Thus, differences in stretch during mounting the tissue may be responsible for the different passive permeabilities reflected by the data in Tables I and II. Since the same investigator obtained all of the flux data, with the exception of those given in Table II, all subsequent discussion of transepithelial fluxes will refer to the data given in Table I.
absorption was further reduced but at no time was secretion of these ions observed.

**Unidirectional Influxes of Na and Cl**

The unidirectional influxes of Na and Cl in the presence of a normal bathing medium and in the absence of either Na or Cl are given in Table III. The influxes of Na and Cl do not differ significantly in the presence of the normal electrolyte medium. Na influx is reduced by approximately 12–13 μmol/cm² h when the mucosal solution is rendered Cl free. Likewise, Cl influx is inhibited by the same amount when the mucosal solution is rendered Na free. In addition, the decrements of Na and Cl influxes resulting from coion replacement do not differ significantly from the net flux of each ion across the tissue under control conditions (Table I). Thus, one site of interaction between the mucosa-to-serosa fluxes of Na and Cl appears to be localized to the mucosal membrane where a similar amount of the unidirectional influx of each ion is dependent upon the simultaneous presence of the other ion.

Sallee et al. (18) have suggested that the presence of unstirred layers adjacent to the mucosal surface may lead to an overestimate of unidirectional influxes because of the failure of the extracellular marker (in this case, [³H]inulin) to fully equilibrate with the mucosal extracellular space during brief exposures. The data given in Tables I and III indicate that the influxes determined using the present techniques cannot be significantly overestimated because $J_{me}^I$ in the absence of Cl (Table III) does not differ significantly from $J_{me}^{Na}$ (Table I). Since $J_{me}$ cannot be less than $J_{ms}$ and because $J_{ms} \geq J_{sm}$ it follows that $J_{me}$ cannot be less than $J_{sm}$. Thus, the possibility that our techniques significantly overestimate $J_{me}$ is excluded.

As shown in Tables I and III, whereas $J_{me}^{Na}$ in the absence of Cl is equal to $J_{sm}^{Na}$, $J_{me}^{Cl}$ in the absence of Na significantly exceeds $J_{sm}^{Cl}$. This finding together with previous suggestions that an exchange diffusion process contributes to the unidirectional transepithelial Cl fluxes (1, 2) prompted an investigation of the effect of intracellular Cl depletion on $J_{me}^{Cl}$. Tissues were preincubated for 1 h
in flasks containing either the Na-free (choline chloride) medium or a Na-free plus Cl-free (mannitol) medium. Chloride influx ($J_{\text{cl}}^\text{in}$) was then determined using Na-free test solutions. When the tissues were preincubated in the Na-free, Cl-containing solution, $J_{\text{cl}}^\text{in}$ averaged $22.4 \pm 1.2 \mu\text{mol/cm}^2\text{h} (n = 9)$, a value that does not differ significantly from that given in Table III for influx in the absence of Na. However, when the tissue was preincubated in Na-free plus Cl-free solutions, $J_{\text{cl}}^\text{in}$ averaged $14.2 \pm 0.9 \mu\text{mol/cm}^2\text{h} (n = 9)$, a value that is significantly lower than that observed when the tissue was preincubated in a Na-free solution that contained Cl. Thus, $8.2 \pm 1.5 \mu\text{mol/cm}^2\text{h}$ of $J_{\text{cl}}^\text{in}$ in the absence of Na appears to be dependent upon whether or not the preincubation solution contained Cl and, by inference, on the intracellular Cl concentration. Inasmuch as these determinations of $J_{\text{cl}}^\text{in}$ were carried out using Na-free solutions, this apparent dependence of a fraction of $J_{\text{cl}}^\text{in}$ on the intracellular Cl concentration (a trans-concentration effect) does not involve the Na-dependent Cl influx but may reflect the presence of a Na-independent Cl-Cl exchange process at the mucosal membrane. Needless to say, there are other possible undetermined factors that could be responsible for the decrease in $J_{\text{cl}}^\text{in}$ after preincubation in a Cl-free medium (i.e. changes in the intracellular electrical potential), however, as will be discussed below, the implication of an exchange diffusion process is supported by the totality of previous and present data.

A series of experiments was performed examining the effect of 1 mM KCN on $J_{\text{cl}}^\text{in}$. Under control conditions, Cl influx averaged $31.4 \pm 2.6 \mu\text{mol/cm}^2\text{h}$ and influx into paired tissues that were preincubated for 30 min in the presence of 1 mM KCN averaged $32.6 \pm 2.0 \mu\text{mol/cm}^2\text{h} (n = 10)$; these values do not differ significantly from each other or from the control value for $J_{\text{cl}}^\text{in}$ given in Table III. Thus, inhibition of aerobic metabolism does not affect $J_{\text{cl}}^\text{in}$. Further, since these experiments were carried out in the presence of Na, the absence of a direct link between Cl influx and aerobic metabolic energy applies to the Na-dependent (coupled) and Na-independent fractions of $J_{\text{cl}}^\text{in}$. These findings are of further interest inasmuch as Zerahn (19) has raised the possibility that reported values of unidirectional Na influx across the outer surface of frog skin may be, in part, a measure of Na that has already moved across at least one layer of transporting cells (i.e. some of the Na taken up by the skin across the outer surface during a brief exposure to radioactive Na has already been subject to active transcellular transport). The possibility that our influx measurements reflect, to a significant degree, transepithelial ion movements can be ruled out since cyanide rapidly abolishes NaCl absorption (3, 20, and unpublished observations) but has no effect on Cl influx.

**Effect of a Transmural PD on the Serosa-to-Mucosa Flux of Chloride**

The finding that $J_{\text{cl}}^\text{in}$ is reduced in tissues that were preincubated in a Cl-free medium together with previous data suggesting that exchange diffusion con-
tributes to the transepithelial fluxes of Cl (2, 3) prompted a series of studies to
determine the effect of an imposed transepithelial PD on \( J_{\text{sm}}^{\text{Cl}} \). In each of six
tissues, \( J_{\text{sm}}^{\text{Cl}} \) was determined under open-circuit conditions (i.e. \( \psi_{\text{ms}} \cong 0 \)) and
when the tissue was clamped at a \( \psi_{\text{ms}} = 35 \) mV, mucosal solution positive.
When \( \psi_{\text{ms}} \cong 0 \), \( J_{\text{sm}}^{\text{Cl}} \) averaged \( 13.5 \pm 0.5 \) \( \mu \)mol/cm²h, a value that is in ex-
cellent agreement with that reported in Table I. When \( \psi_{\text{ms}} = 35 \) mV, \( J_{\text{sm}}^{\text{Cl}} \)
averaged \( 22.1 \pm 0.8 \) \( \mu \)mol/cm²h. This marked increase in response to an
imposed PD indicates that a large fraction of \( J_{\text{sm}}^{\text{Cl}} \) can be attributed to simple
ionic diffusion. More precisely, it can be readily shown that (11, 21).

\[
J_{\text{sm}}^{\text{Cl}} = \phi(J_{\text{sm}}^{\text{Cl}}[\frac{\xi}{(\exp \xi - 1]}] + \mu J_{\text{sm}}^{\text{Cl}} \tag{1 a}
\]

and, when \( \psi_{\text{ms}} = 0 \)

\[
J_{\text{sm}}^{\text{Cl}} = \phi J_{\text{sm}}^{\text{Cl}} + \mu J_{\text{sm}}^{\text{Cl}} \tag{1 b}
\]

where \( J_{\text{sm}}^{\text{Cl}} \) is the total serosa-to-mucosa flux in the presence of any \( \psi_{\text{ms}} \); \( \phi J_{\text{sm}}^{\text{Cl}} \) is
the diffusional flux of Cl from serosa-to-mucosa when \( \psi_{\text{ms}} = 0 \); \( \mu J_{\text{sm}}^{\text{Cl}} \) is a non-
diffusional, PD-independent contribution to \( J_{\text{sm}}^{\text{Cl}} \); and \( \xi = \frac{F \psi_{\text{ms}}}{RT} \). Substituting
the above values into Eq. 1 a, b, we find that \( \phi J_{\text{sm}}^{\text{Cl}} = 11.3 \) \( \mu \)mol/cm²h and
\( \mu J_{\text{sm}}^{\text{Cl}} = 2.2 \) \( \mu \)mol/cm²h. Thus, 84\% of the total \( J_{\text{sm}}^{\text{Cl}} \) under open-circuit conditions can be attributed to simple ionic diffusion; as will be discussed further
below, the remainder (\( \mu J_{\text{sm}}^{\text{Cl}} \)) appears to be attributable to exchange diffusion.

**Apparent Electrochemical Potential Differences**

The electrical potential difference across the mucosal membrane (\( \psi_{\text{me}} \)) of
rabbit gallbladder cells, with reference to the mucosal solution, averaged
\( 45 \pm 1 \) mV (cell interior negative) in a total of 115 impalements on gall-
bladders from seven animals. Interanimal variation in \( \psi_{\text{me}} \) was small and
mean values obtained from different gallbladders did not differ significantly.
Typical recordings of \( \psi_{\text{me}} \) obtained from three impalements are illustrated in
Fig. 2. The transmucosal potential difference did not decline significantly

![Figure 2](attachment:figure2.png)

**Figure 2.** Tracings of typical recordings of the intracellular electrical potential with
respect to the mucosal solution (\( \psi_{\text{ms}} \)) under control conditions.
during at least 2 h of incubation. The average value for $\psi_{mc}$ given above is in excellent agreement with that reported by Hénin et al. (quoted in reference 8). van Os (8) reported a mean value of $-69.3$ mV for the intracellular potential in rabbit gallbladder but he excluded values smaller than $-50$ mV and the average represents only 16% of all impalements. In tissues incubated in Na-free solutions (Na replaced with choline), there was little deviation of $\psi_{mc}$ from the values obtained in the presence of the normal buffer; the mean value of 85 impalments on tissues from seven animals in the Na-free solution was $-41 \pm 1$ mV.

Intracellular Na, K, and Cl concentrations determined on mucosal strips

<table>
<thead>
<tr>
<th>Ion</th>
<th>Extracellular concentration</th>
<th>Calculated cell concentration</th>
<th>Measured cell concentration</th>
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<tbody>
<tr>
<td>Na</td>
<td>140</td>
<td>350</td>
<td>66±3</td>
</tr>
<tr>
<td>Cl</td>
<td>145</td>
<td>30</td>
<td>84±3</td>
</tr>
<tr>
<td>K</td>
<td>12</td>
<td>70</td>
<td>85±7</td>
</tr>
</tbody>
</table>

| Dry weight per wet weight ratio = 0.126±0.004 |
| Extracellular space (A/mg wet weight) = 0.306±0.022 |
| Intracellular water (mg/mg wet weight) = 0.57±0.03 |

Each value for the intracellular concentration represents the mean ± SEM of duplicate determinations on four tissues. The values for the dry weight per wet weight ratio and the relations between the intracellular and extracellular spaces and the wet weight are the results of data obtained on 12 tissues. The "calculated cell concentration" are the values predicted by the Nernst equation for a passive distribution given the extracellular concentrations and an intracellular electrical potential of $-45$ mV with respect to the external solutions.

are given in Table IV. Like many other cell types, gallbladder epithelium is characterized by a higher intracellular K concentration and a lower intracellular Na concentration than those present in the bathing media. The values of [Na]_c and [K]_c given in Table IV do not differ significantly from those reported by van Os for rabbit gallbladder (8) or by Diamond (1) for fish gallbladder. The intracellular Cl concentration is somewhat higher than that observed in most tissues but is in good agreement with that reported by Diamond for fish gallbladder (1). The second column of Table IV gives the Na, K, and Cl concentrations of the normal bathing media employed in these studies. The third column shows the intracellular concentrations that would be predicted for a passive distribution of each ion between the intracellular and extracellular compartments calculated using the Nernst equation assuming an intracellular electrical potential of $-45$ mV and equal intracellular and extracellular ionic activities. Despite the inherent uncertainties in interpreting overall intracellular ion concentrations, the data in Table IV suggest that Na is actively extruded from the cells and that Cl is actively accumulated.
against an electrochemical potential difference. The equilibrium value predicted for the intracellular K concentration does not differ markedly from the measured $[K]_e$.

The effect of incubation in Na-free media on intracellular Cl concentration is illustrated in Fig. 3. Half of each bladder strip served as a control and was incubated in the normal (Na-containing) buffer. Control values obtained at either 30- or 60-min incubation did not differ significantly. In contrast, tissues incubated in Na-free media showed a progressive decline of $[Cl]_e$ toward the value expected for electrochemical equilibrium. The curve shown is given by

$$[Cl]_e = 64 \exp \left(-0.023t\right) + 31,$$

where $t$ is the time of incubation in the Na-free buffer in hours, and $[Cl]_e$ when $t = \infty$ (31 mM) is the calculated value for electrochemical equilibrium; this curve adequately describes the experimental data. Thus, these data suggest that the presence of Na in the bathing media is necessary for accumulation of intracellular Cl against an apparent electrochemical potential difference.

**Transepithelial Fluxes of Sulfate**

In eight experiments $J_{in}^{SO_4}$ and $J_{in}^{Cl}$ were determined on paired tissues from the same gallbladders. $J_{in}^{Cl}$ was determined in the presence of the normal elec-
trolyte solution whereas $J_{SO_4}^{in}$ was determined using the Cl-free solution (70 mM SO$_4$). $J_{SO_4}^{in}$ averaged $11.4 \pm 0.69 \mu$mol/cm$^2$h ($n = 8$), a value that is in good agreement with that reported in Table I. $J_{SO_4}^{in}$ averaged only $0.72 \pm 0.04 \mu$mol/cm$^2$h, indicating that the tissue is only slightly permeable to SO$_4$.

CONCLUSIONS

"Neutral" or "Electrically Coupled" NaCl Transport?

Diamond (1) concluded from his studies on fish gallbladder in vitro that the active absorption of Na and Cl is the result of a coupled electrically neutral transport process. This conclusion was based primarily on the findings that active NaCl transport from the mucosal solution to a serosal solution having an identical composition was not associated with a significant transepithelial PD and that replacing Na or Cl with nontransported ions abolished NaCl and fluid absorption without affecting the PD. Both of these characteristics differ from those of epithelia such as isolated frog skin, where Cl transport is passively driven by a transepithelial PD resulting from active Na transport ("electrical coupling"). Later, Wheeler (2), Dietschy (4), and Diamond (3) extended these findings to a sac preparation of in vitro rabbit gallbladder. The findings reported in Table I confirm these observations inasmuch as under control conditions the rates of net Na and Cl absorption were equal in the absence of a significant PD ($< \pm 1$ mV) and replacement of either Na with choline or Cl with SO$_4$ abolished net movements of both Na and Cl without affecting the PD. Indeed, the net fluxes of Na and Cl determined in the present study using isotope techniques are in excellent agreement with the chemical determinations reported by Wheeler (2). In the latter study, net Na absorption under control conditions averaged 33 $\mu$mol/h, 100 mg wet weight and the estimated surface area corresponding to 100 mg wet weight of tissue was 2.5 cm$^2$ so that the rate of Na absorption was $\sim 13 \mu$mol/cm$^2$h; this value is in excellent agreement with the value of $13.9 \pm 2.1 \mu$mol/cm$^2$h found in the present study (Table I). In this respect, it is of interest that Whitlock and Wheeler (22) observed net NaHCO$_3$ absorption of approximately 1 $\mu$mol/cm$^2$h from a Cl-free medium containing 25 mM HCO$_3$; thus, the low concentration of HCO$_3$ (10 mM) present in our Cl-free medium may be responsible for the residual net Na absorption of $0.7 \pm 0.3 \mu$mol/cm$^2$h given in Table I.

Although at face value the findings reported by Diamond (3), Dietschy (4), Wheeler (2), Whitlock and Wheeler (22), and confirmed in the present study suggest a direct interaction between Na and Cl transport as opposed to electrical coupling, the possibility has been raised that the coupling between Na and Cl absorption may, indeed, be electrical and that the absence of a significant transepithelial PD may be due to:

(a) Active transport of Na into an intraepithelial compartment, such as the lateral intercellular spaces, that is bounded by highly conductive plasma
membranes or extracellular pathways and, therefore, possesses a short electrical space (or length) constant. Under these conditions, a local intraepithelial PD may be established by active Na transport that serves as the driving force for passive Cl absorption, but this PD could be dissipated within the epithelium so that no significant transepithelial PD is discernable using external electrodes. This intriguing model, suggested by Keynes (6) and formally developed by Hille and Hille (23) is closely analogous to the “standing osmotic gradient” model (24) for isotonic fluid absorption in which a local osmotic gradient, established within the intercellular spaces, is dissipated by osmotic water flow so that the emergent fluid is isotonic. Similarly, an electrical potential difference between the mucosal solution and the intercellular space could be dissipated by current (ion) flow between the cell interior, or the mucosal solution, and the interspace.

(b) Moreno and Diamond (footnote 3, reference 7) and van Os (8) have recently raised the possibility that the absence of a significant PD across rabbit gallbladder during spontaneous NaCl absorption and when either Na or Cl are replaced with nontransported ions may be attributed to high conductance paracellular shunt pathways which would attenuate any electromotive force developed by ion pumps in the transcellular pathway. However, this suggestion was not supported by quantitative considerations (see below).

The possibility that Cl absorption is electrically (passively) coupled to active Na absorption is also suggested by the findings that: (a) Gallbladders from several other species are characterized by significant spontaneous transepithelial PD’s (up to 8 mV) which appear to be directly related to the transepithelial resistance of the tissue (25, 26). (b) Rabbit gallbladder possesses a ouabain-sensitive Na-K-dependent ATPase which has been implicated in active Na absorption by a number of epithelia for which there is no compelling evidence for a neutral NaCl absorptive process. Further, NaCl absorption by rabbit gallbladder appears to be directly or indirectly linked to this ATPase activity insomuch as transport is inhibited by ouabain (4, 8, 27) and, at least according to some investigators, by removal of K from the serosal solution (28). (c) Whereas the cation transport mechanism in rabbit gallbladder appears to be highly specific for Na, a variety of monovalent anions (including acetate, bicarbonate, lactate, pivalate, propionate, isethionate, and 5,5-dimethyl-2,4-oxazolidinedione [DMO]) are at least partially effective in replacing Cl with respect to sustaining fluid transport in the presence of Na (22). This apparent lack of anion specificity is highly uncommon for carrier-mediated transport processes and suggests a more nonspecific mechanism for the coupling between Na and anion absorption.

A Working Model of Rabbit Gallbladder

The results of the present study strongly suggest that the notion that “neutral transport” and “electrical coupling” are mutually exclusive alternatives is in-
correct and stems from a conceptual framework that views the gallbladder as a single membranous barrier. These results can perhaps be best summarized in the form of the working model illustrated in Fig. 4. According to this model:

(a) The mucosal membrane possesses a mechanism capable of mediating the neutral influx of NaCl from the mucosal solution into the absorptive cells. In the presence of 140 mM NaCl, influx via this mechanism amounts to approximately 14 μmol/cm²h; however, replacement of Na with choline or Cl with SO₄ completely inhibits this process (Table III). Hence, Na and Cl entry into the cells are obligatorily linked at the mucosal membrane. Since the

rate of coupled NaCl influx across the mucosal membranes does not differ significantly from the rate of active transepithelial NaCl transport, NaCl efflux from the cell across the mucosal membrane, within the limits of experimental error, does not appear to differ significantly from zero.² Finally, as discussed previously, this mechanism appears to be capable of bringing about the net transport of Cl from the mucosal solution into the cell against an electrochemical potential difference; a possible source of the energy for this "uphill" movement will be discussed below.

(b) In addition, the mucosal membranes appear to possess a mechanism capable of mediating exchange diffusion of Cl between the mucosal solution and the cell interior. Previous studies have suggested, on the basis of indirect evidence, that the transepithelial fluxes of Br and Cl in fish (1) and rabbit (2) gallbladder are complicated by exchange diffusion. The present studies confirm this notion and localize the responsible mechanism to the mucosal membr-

² It seems highly unlikely that the coupled influx process is irreversible; instead, it seems more probable that influx is slightly underestimated by backflux during the brief exposure so that \( J_{\text{m}+} > J_{\text{cm}+} \).
brane. Under control conditions, the magnitude of the obligatory Cl:Cl exchange is at least 8 μmol/cm²h.

(c) At the serosal membrane, Na is actively extruded from the cell into the serosal solution, against a steep electrochemical potential difference, at a rate of 14 μmol/cm²h. In view of the fact that a ouabain-sensitive, Na-K ATPase has been identified in rabbit gallbladder and that the activity of this enzyme has been correlated with the rate of transepithelial NaCl transport (8, 29), it seems reasonable to infer that active Na extrusion at the serosal membrane is mediated by this ATPase and is, in all likelihood, coupled to K uptake by the cell.

(d) The apparent electrochemical potential of intracellular Cl is considerably greater than that in the serosal solution so that Cl exit from the cell may be entirely diffusional; at present there is no need to invoke a more complex mechanism. The finding that when Na is replaced with choline net transepithelial Cl transport and the apparent electrochemical potential difference of Cl between the cell interior and the serosal solution are abolished is consistent with this notion. Thus, in the absence of Na, the driving force for Cl entry across the mucosal membrane (cotransport with Na) and for Cl exit across the serosal membrane (the electrochemical potential difference) are both abolished. Accordingly, using a net flux of 14 μmol/cm²h, the intracellular and extracellular Cl concentrations shown in Fig. 4 and an electrical potential difference of 45 mV between the cell interior and the serosal solution, we have calculated the bidirectional diffusional fluxes of Cl across the serosal membrane consistent with the Ussing flux-ratio equation (30); these values are given in Fig. 4.

(e) The diffusional fluxes of Na and Cl through the shunt pathway given in Fig. 4 were calculated from the relations (12)

\[ J_{n,m} = \frac{J_{m,e} J_{e,s}}{(J_{m,e} + J_{e,s})} + \alpha J_{m,e} \]  
\[ J_{e,m} = \frac{J_{m,e} J_{e,s}}{(J_{m,e} + J_{e,s})} + \alpha J_{e,m} \]

3 Preincubation in the Cl-free medium may not have completely depleted [Cl]c so that some exchange diffusion component may have still contributed to \( J_{e,m} \).

4 Currently, the partial ionic conductance of Cl (GCl) across the basolateral membranes of rabbit gallbladder is unknown. Frömling (31) has reported that the total conductance of the basolateral membranes of Necturus gallbladder is 0.34 mho/cm². If a similar value obtains for rabbit gallbladder and if the entire conductance is attributable to Cl movements, the net diffusional flux of Cl across the basolateral membranes can be calculated from

\[ J_{\text{net}} = G_{\text{Cl}} (E_{\text{Cl}} + \psi_{\text{es}})/F. \]

where \( E_{\text{Cl}} = (RT/F) \ln ([\text{Cl}]_{i}/[\text{Cl}]_{o}) \). Using the data in Table IV and a value for \( \psi_{\text{es}} \) of 45 mV, the calculated value for \( J_{\text{net}} \) is only 0.3 μmol/cm²h. However, unlike Necturus gallbladder, rabbit gallbladder is a villous structure. The extent to which the villi increase the area of the epithelial cell layer per unit serosal area is uncertain, however, for small intestine this has been estimated to be 30- to 40-fold. This would increase the calculated \( J_{\text{net}} \) to a value of 9-12 μmol/cm²h which does not differ markedly from the net flux reported in Table I. Currently available data do not permit us to exclude a diffusional exit of Cl from the cell across the basolateral membranes of rabbit gallbladder.
where $m$, $e$, and $s$ designate the mucosal, intracellular, and serosal compartments, respectively, and $J_{ij}$ is the unidirectional flux from the $i$th compartment to the $j$th compartment. $\delta J_{ij}$ represents the unidirectional diffusional fluxes through the shunt or extracellular pathway. Clearly, when the mucosal and serosal solutions have identical compositions and the transepithelial PD is close to zero, $\delta J_{ms} \approx \delta J_{sm}$. Using the values for $J_{ms}$, $J_{em}$, $J_{es}$, and $J_{se}$ shown in Fig. 4, the calculated contributions of the shunt pathway to transepithelial Na and Cl fluxes are 21 $\mu$mol/cm²h and 11 $\mu$mol/cm²h, respectively. The value for Cl diffusion through the shunt pathway is in excellent agreement with that determined independently from the effect of $\psi_m$ on $J_{esc}$; the total contribution of the mucosal Cl-Cl exchange diffusion process to $J_{ms}$ and $J_{es}$ is only $\sim 2$ $\mu$mol/cm²h.

The conductance of the shunt pathway due to the diffusional movements of Na and Cl is 33 mmho/cm². The total tissue conductance in these experiments averaged $\sim 35$ mmho/cm² (as noted above, it is difficult to determine this value precisely because of the very large correction that must be made for fluid resistance between the tissue and the agar bridges used to monitor the PD resulting from an imposed current). Thus, the shunt conductance accounts for approximately 95% of the total tissue conductance. Wright et al. (17) have reported a value of 36 ± 1 mmho/cm² for the conductance of rabbit gallbladder bathed by solutions containing 150 mM Na and have argued that at least 95% of the total conductance can be attributed to the shunt pathway. Further, Frömter (31) has shown that 96% of the total conductance across *Necturus* gallbladder can be accounted for by the conductance of the shunt pathway. Our data and analysis are entirely consistent with these findings.

Finally, according to the data shown in Fig. 4, $P_{Cl}/P_{Na}$ for the shunt pathway is equal to 0.5. Barry et al. (32) have presented evidence that $P_{Cl}/P_{Na}$ in rabbit gallbladder is time dependent and varies from a very low value (0.08 or less) a few minutes after dissection of the gallbladder to 0.33 after 1 h. These authors have postulated that this time dependence is due to “the development of something approximating a free solution shunt” after dissection. It should be stressed that these investigators employed a sac preparation whereas our studies were performed on flat sheets stretched and mounted between Two lucite half-chambers; this difference may explain why the value of $P_{Cl}/P_{Na}$ in the present studies (0.5) is somewhat greater than that observed by Barry et al. (0.33) (32). We did not note any systematic increase in bidirectional Cl fluxes with time, however, all of our flux determinations commenced after at least 45 min had elapsed after dissection. Thus, it is quite possible that, for whatever reason, the increase in the anion permeability of the tissue had already taken place in our preparation by the time our earliest measurements were obtained.
The working model illustrated in Fig. 4 is quantitatively consistent with all of the present data and satisfactorily accommodates most previous observations on fish and rabbit gallbladder. Thus:

(a) Net transepithelial transport of both Na and Cl take place in the absence of transepithelial electrochemical potential differences (i.e. under “self-shorted-circuited” conditions) and, therefore, may be considered “active transport” processes (see below). However, the sites at which energy is invested into the uphill movements of these ions differ.

(b) Equal rates of transcellular Na and Cl absorption are assured, under steady-state conditions, by a mechanism located at the mucosal membrane that brings about one-for-one entry of Na and Cl into the cells.

(c) Replacement of Na or Cl with a nontransported ion abolishes net transport of both ions. In view of the finding of Whitlock and Wheeler (22) that a host of monovalent anions can sustain coupled Na-anion absorption, additional studies are necessary to define the anion specificity of the coupled influx mechanism.

(d) Finally, it should be noted that within the framework of this model, the distinction between neutral transport and electrical coupling is not meaningful. Whereas, Cl enters the cell coupled to Na entry by means of a neutral mechanism, Cl exit may be diffusional. If the latter notion is correct, part of the driving force for this movement is the electrical potential difference across the serosal membrane.

**Electrophysiologic Considerations**

Much of the debate concerning the question of neutral vs. electrical coupling appears to center about the absence or presence of a significant transepithelial PD during the course of ion transport from one solution to another having an identical composition. The fact that this is an inappropriate criterion for distinguishing between these modes of transport can best be demonstrated by considering a previously published (13, 21) equivalent electrical circuit model, which is illustrated in Fig. 5. Here, $E_m$ is an electromotive force across the mucosal membrane, $R_1$ is the internal resistance of this battery and $R_2$ is a shunt resistance across that membrane; $E_1$, $R_3$ and $R_4$ and $E_L$, $R_5$ and $R_6$ are the analogous parameters of the serosal membrane and permselective shunt pathway, respectively. The solution of this circuit for the transepithelial PD ($\psi_{m,s}$) with respect to the mucosal solution is:

$$\psi_{m,s} = [(E_1 R_1 - E_m R_m) R_3 R_L/R_1] + [E_2 R_2 (R_1 R_M + R_5 R_6) / R_1],$$

where $R_m = R_3/(R_1 + R_3)$; $R_s = R_4/(R_3 + R_4)$; $R_L = R_6/(R_3 + R_6)$, and $R_1 = R_2 R_m + R_3 R_s + R_5 R_L$.

Now, with reference to the models illustrated in Figs. 4 and 5, there is no a
priori reason why $E_mR_m$ should equal $E_sR_s$ even if there is a neutral cotransport mechanism for an anion and a cation at one membrane. The permselective properties of the mucosal and serosal membranes certainly need not be identical so that diffusion potentials across these two membranes will, in general, differ. Further, if either or both membranes possess rheogenic carrier-mediated processes, they would contribute to $E_m$ and/or $E_s$. Thus, in general, the finding that $\psi_{m*} \cong 0$ when $E_sR_s \cong E_mR_m$ must mean that $R_s/R_s/R_m$ is small. As discussed above, for rabbit gallbladder $R_sR_m \cong 0.05 (R_sR_m + R_sR_s)$ so that when $E_sR_s \cong 0$

$$\psi_{m*} \cong 0.05 (E_sR_s - E_mR_m).$$

That is, the difference between the two electromotive forces across the limiting membranes is markedly attenuated because the shunt resistance ($R_sR_s$) is relatively low compared to the resistance of the transcellular pathway ($R_sR_m + R_sR_s$). Under these conditions, $\psi_{m*}$ will be small and linearly related to the resistance of the shunt pathway (which is essentially equal to the resistance of the total tissue). Further, in the presence of ionic gradients across the permselective pathway, $\psi_{m*}$ will be dominated by electromotive forces arising in the shunt ($E_sR_s$). Under these conditions, the attenuated contribution from ($E_sR_s - E_mR_m$) may be masked entirely.

It should be stressed that the presence of a low or insignificant $\psi_{m*}$ across any epithelium, assuming that in general $E_sR_s \neq E_mR_m$, must be due to a

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Figure 5. A general equivalent electrical circuit model for epithelial tissues. From (13).
low value of $R_3R_L/R_i$. However, this does not imply that the presence of a shunt pathway with a low absolute resistance is sufficient. A low $\psi_{ms}$ can be attributed to a low shunt resistance alone, only if the conductance of the shunt (or passive conductance pathway) is sufficient to permit the open-circuit PD (generated by active ion transport) to drive passive (diffusional) ionic movements at the experimentally observed rate of net ion flow. This condition defines pure electrical coupling. If the conductance of the shunt pathway is insufficient, then the low $\psi_{ms}$ cannot be attributed to a low value of $R_3R_L$ alone, pure electrical coupling can be excluded, and a mechanism(s) capable of bringing about the active transepithelial transport of cation and anion (i.e. neutral but not necessarily coupled transport) must be invoked. The present data permit an explicit evaluation of the possibility that the low $\psi_{ms}$ across rabbit gallbladder is entirely attributable to the presence of a low resistance shunt pathway; that is, whether the transepithelial transport of either Na or Cl is purely electrically coupled to the active transport of the other.

It can be readily shown that for small values of $\psi_{ms}$ (< 25 mV) the net diffusional flow of an ion, $i$, through transepithelial shunt and/or passive conductance pathways is given by (33)

$$\dot{J}_{net} = P_i ([i]_m \exp (z_i F \psi_{ms}/RT) - [i]_s \exp (z_i F \psi_{ms}/RT)),$$

(5)

where $P_i$ is the permeability of the diffusional pathway to $i$, and the bracketed terms designate the concentrations of $i$ in the mucosal ($m$) and serosal ($s$) solutions. When $[i]_m = [i]_s$, Eq. 5 reduces to

$$\dot{J}_{net} = -P_i [i]_m z_i F \psi_{ms}/RT.$$

(6)

Now, when $\psi_{ms} = 0$ (i.e. under short-circuit conditions), $P_i [i]_m = \sigma J_{ms}$ (or $\sigma J_{an}$) which when expressed in umol/cm²h is essentially numerically equal to the partial ionic conductance of $i$ ($G_i$) expressed in millimhos per square centimeter. Thus, we may write

$$\dot{J}_{net} = \pm G_i F \psi_{ms}/RT.$$

(7)

The "shunt" implies a paracellular pathway whereas the term "passive conductance pathway" includes transcellular and extracellular routes for ionic diffusion. However, as discussed above, in leaky epithelia such as rabbit gallbladder virtually all transepithelial ionic diffusion traverses the paracellular route.

"Short-circuited" isolated frog skin is a classical extreme example of a situation in which the zero transepithelial PD can be attributed entirely to (the artificial insertion of) a high (infinite) conductance shunt pathway. Clearly, the conductance of the shunt is sufficient to permit the passive movement of Cl at a rate equal to the net movement of NaCl; this establishes pure electrical coupling between the movements of Na and Cl.

$\sigma J_{ms}$ is the diffusional flow of $i$ from mucosa to serosa under short-circuit conditions and is clearly equal to $\sigma J_{in}$. 
Since a partial ionic conductance ($G_i$) cannot exceed the total transepithelial conductance ($G$), it can be readily shown that even if $G_{cl} = G = 40$ mmho/cm$^2$, $\psi_m$, would have to be 10 mV, serosa positive, for $J_{cl}^{in}$ to be purely electrically coupled to active Na absorption at a rate of 14 $\mu$mol/cm$^2$h. Conversely, if only Cl is actively transported and if $G_{Na} = G = 40$ mmho/cm$^2$, $\psi_m$, would have to be 10 mV serosa negative to account for passive Na absorption at a rate of 14 $\mu$mol/cm$^2$h. The values of $\psi_m$, observed in this study were between $-1$ mV, in agreement with previous data on rabbit gallbladder; it is conceivable that technical errors could have “masked” a PD of $\pm 10$ mV or could have resulted in a ninefold underestimate of $G$ (i.e. $G_{cl}$ would have to be 369 mmho/cm$^2$ to permit 1 mV to drive a diffusional movement of Cl equal to 14 $\mu$mol/cm$^2$h).

Thus, in spite of the fact that the total tissue conductance is very large, it is nonetheless grossly insufficient to permit pure electrical coupling of passive Cl transport to active Na transport or passive Na transport to active Cl transport at the observed rates even if the entire tissue conductance is assigned to the passively driven ion. It follows that the low $\psi_m$, cannot be due to the low resistance shunt alone, and this conclusion does not depend on the validity of the calculated partial ionic conductances given in Fig. 4. Since, in fact, $G_{cl} < G_{Na} < G$, the values for $\psi_m$, necessary for pure electrical coupling of the passive movement of either ion to the active movement of the other would be considerably greater than $\pm 10$ mV. Our data indicate that $\psi_m = G_{cl} = 11$ mmho/cm$^2$; thus, $\psi_m$, would have to be 34 mV (serosa positive) for pure electrical coupling of net Cl transport to active Na transport. Further, if the “native” (undissected) gallbladder is characterized by a very low $G_{cl}$ as reported by Barry et al. (32), active Na absorption would have to be associated with a very large PD in situ.

Thus, these considerations essentially exclude the possibility of pure electrical coupling between the movements of Na and Cl driven by a transepithelial PD. We must now consider the possibility, raised by Keynes (6), that net Cl absorption is electrically coupled to active Na transport but that the required PD is intraepithelial and is dissipated by local currents so that the transepithelial PD is negligible. If so, replacement of Cl with the relatively impermeant, nontransported anion, SO$_4$, should result in an increase in this local PD (serosal direction positive) and the abolition of net Na transport should be in part due to an increased backflux of Na. That is, the active Na transport mechanism would deliver Na into a region of increased relative electrical positivity which, in turn, would provide the driving force for the passive backflux of Na; the local ionic currents responsible for the dissipation of the intraepithelial PD would of necessity involve the recycling of Na. However, as shown in Table I, replacement of Cl with SO$_4$ abolishes net Na absorption through a reduction in $J_{Na}^{in}$ alone. If the mechanism suggested by Keynes were operative,
an increase in $J_{Na}^\text{mut}$ should have been in part responsible for the decrease in $J_{Na}^\text{mut}$. Assuming that the rate of active Na transport from mucosa to serosa is essentially unaffected by a small increase in $\psi_m$, the abolition of $J_{Na}^{\text{mut}}$ (14 $\mu$mol/cm$^2$h) should have been the combined result of a decrease in $J_{Na}^{\text{mut}}$ of approximately 7 $\mu$mol/cm$^2$h and an equivalent increase in $J_{Na}^\text{mut}$; the observed value of $J_{Na}^\text{mut}$ and $J_{Na}^\text{mut}$ in the presence of a Cl-free, $SO_4$ medium, $\sim$22 $\mu$mol/cm$^2$h, differs markedly ($P < 0.001$) from the value of $\sim$29 $\mu$mol/cm$^2$h, which would be predicted by the model suggested by Keynes. The same argument can be employed to exclude an independent active Cl transport mechanism with electrically coupled passive Na absorption since replacement of Na with choline abolished $J_{Cl}^{\text{mut}}$ solely by a reduction in $J_{Cl}^{\text{mut}}$. Thus, although the effects of replacement of Cl with $SO_4$ or Na with choline on the bidirectional fluxes of Na and Cl are entirely consistent with the model illustrated in Fig. 4, they cannot be readily reconciled with a model that involves purely passive movement of one ion coupled electrically to the active transport of the other via an intraepithelial PD.

These considerations appear to exclude the possibility of pure electrical coupling between the movements of Na and Cl across rabbit gallbladder and, thus, necessarily imply the presence of a neutral, coupled transport mechanism. In terms of the equivalent electrical circuit model, this means that the low value of $R_xR_L$ is insufficient, by itself, to account for the fact that net NaCl transport at a rate of 14 $\mu$mol/cm$^2$h is associated with a $\psi_m$ of only $\pm$1 mV. It follows that the low PD must be, at least in part, a consequence of a highly resistive transcellular pathway, which in turn is the result of the presence of a neutral, coupled transport mechanism for Na and Cl. Clearly, a neutral, coupled influx process permits the nonconductive entry of the two major extracellular ions into the cell across the mucosal membrane. This must increase $R_xR_m$ above the value that would be observed if either Na or Cl simply diffused across the mucosal membrane, and, in turn, must increase the relative resistance of the transcellular pathway with respect to the shunt pathway. Stated otherwise, the relatively low conductance of the transcellular route compared to the paracellular route is in part attributable to the nonconductive, coupled influx process.

In summary, the low value of $R_xR_L/R_t$ which must be ultimately responsible for the fact that $\psi_m \equiv 0$ is the combined result of (a) a high value of $R_xR_m$ which is the direct consequence of a nonconductive, coupled NaCl influx process and (b) a low value of $R_xR_L$. The latter, by itself, is entirely inadequate whereas the nonconductive influx process could ensure a low $\psi_m$, even in the presence of a passive conductance pathway(s) with a relatively high absolute resistance. For example, if the mucosal membrane is entirely nonconductive, as illustrated in our incomplete working model, $R_xR_m \to \infty$ and $\psi_m \equiv 0$ when $E_LR_L \equiv 0$ regardless of the resistance of the shunt ($R_xR_L$).
Further, it should be clear that (a) the absence of a significant PD does not necessarily imply the presence of a neutral, coupled transport mechanism, and conversely (b) the presence of a significant PD does not exclude such a process. Such notions are based on the misconception that electrical coupling and neutral transport are mutually exclusive alternatives and derive, in part, from the view that the epithelium can be treated as if it were a single membrane. Clearly, pure electrical coupling and pure neutral transport are two extremes and intermediate situations are readily feasible. For example, if the mucosal membrane of the model illustrated in Fig. 4 is permeable to Cl but relatively impermeable to Na, transcellular Cl absorption would no longer equal transcellular Na absorption. Under these conditions, there would be a significant PD (serosa positive) which would provide the driving force for net diffusion of Na from serosa-to-mucosa through the shunt (thus, decreasing the rate of net Na absorption below the rate of active Na absorption) and for net Cl diffusion from mucosa-to-serosa (partly through the shunt and partly transcellular). The final result, of course, would be one-for-one Na and Cl absorption but only a fraction of the transepithelial movement of Cl would be driven by the transepithelial PD (i.e. electrically coupled to net Na absorption); the remainder would be a nondiffusional transepithelial movement driven by the neutral, coupled entry mechanism. Net Cl absorption would be observed under short-circuit conditions and, under open-circuit conditions, $G_{Cl}$ together with $J_{Na}$ would be insufficient to account for the entire $J_{net}^{Cl}$.

If we extend this line of reasoning further, it is clear that we can arbitrarily assign different Cl permeabilities to the mucosal and serosal membranes of the model illustrated in Fig. 4 and thereby vary the extent to which transepithelial Cl movement is electrically coupled to Na absorption. In the extreme, if the serosal membrane is impermeable to Cl but the mucosal membrane is permeable to Cl, net Cl absorption must be entirely diffusional through the shunt pathway. This movement will be driven by a PD whose magnitude will be determined by $G_{Cl}$ and $J_{net}^{Na}$ and under short-circuit conditions, $J_{net}^{Cl} = 0$. Yet, clearly, replacement of Cl with an ion that is not transported by the coupled influx process would abolish Na transport and the PD; thus, the neutral coupled influx process would still be ultimately responsible for NaCl absorption even though Cl transport is entirely diffusional.

Frömter has found that the PD across Necturus gallbladder is approximately 2 mV, serosa positive (31). Further, Rose et al. (25) have reported that gallbladders from a number of species are characterized by significant PD's (serosa positive). The PD across human gallbladder is approximately 8 mV, and under short-circuit conditions $J_{net}^{Cl} = 0$ and the short-circuit current agrees favorably with $J_{net}^{Na}$. As discussed above, these findings do not by themselves exclude a coupled, neutral influx process and it is entirely possible that
they may be attributed to species differences in the permselective properties of the limiting cell membranes.\footnote{Since the submission of this manuscript, Gelarden and Rose (51) have reported that replacement of Cl\textsuperscript{−} with SO\textsubscript{4} \textsuperscript{−} abolishes the electrical potential difference across goose and monkey gallbladders. This finding is entirely consistent with the presence of a coupled NaCl entry process at the mucosal membrane together with a significant backflux of Cl\textsuperscript{−} from the cell across the mucosal membrane. As discussed above, under these conditions Na entry, transepithelial Na transport, and the transepithelial electrical potential difference would be abolished by replacement of Cl\textsuperscript{−} with a nontransported anion.}

**Energetics of Active Chloride Absorption**

Several characteristics of active Cl transport by rabbit gallbladder resemble the active transport of sugars and amino acids by small intestinal epithelium (34). First, active Cl transport is abolished when Na is replaced by choline. Second, there appears to be a coupled entry of Na and Cl across the mucosal membranes. Third, the electrochemical potential of intracellular Cl appears to exceed that in the surrounding media, so that during the course of absorption net Cl entry into the cell from the mucosal solution is directed against an electrochemical potential difference. Fourth, in the absence of Na, the intracellular exchangeable Cl concentration declines toward that value which would be expected for a passive distribution (Fig. 3). All of these findings suggest that the energy necessary for Cl entry into the cell against an electrochemical potential difference and, ultimately, for active transepithelial Cl transport may be derived at least in part from the electrochemical potential difference of Na across the mucosal membrane via the cotransport mechanism. The apparent electrochemical gradient for Na across the mucosal membrane, calculated from the data given in Table IV, is 1.5 kcal/mol; this is likely to be an underestimate since studies on a variety of cell systems indicate that the cytoplasmic activity of Na is approximately one-half the intracellular concentration (cf. 35). The energy required for the apparent uphill movement of Cl into the cell is 0.7 kcal/mol. Thus, the Na gradient is more than sufficient to drive uphill Cl transport, and the efficiency of energy conversion need be at most 50%.

This notion provides a reasonable explanation for the findings of Martin and Diamond (27) on the energetics of fluid transport by rabbit gallbladder. These authors demonstrated that approximately 25 mol of NaCl were actively transported per mole of O\textsubscript{2} consumed. Comparison of this value with data from studies on other epithelia which transport only Na actively (i.e. 20–25 mol of Na actively transported per mole O\textsubscript{2} consumed) suggested that rabbit gallbladder pumps twice as many ions actively per mole O\textsubscript{2} consumed than do these other epithelia. These findings are consistent with the above argument and the model illustrated in Fig. 4. Thus, according to this model, metabolic
energy is directly linked to the active Na extrusion mechanism at the serosal membranes. This mechanism probably involves a Na-K ATPase similar to that found in other epithelia and there is no reason to suppose that the average stoichiometry between ATP consumption and active Na extrusion by this mechanism should differ among epithelia. Active Cl transport is energized by the Na gradient, resulting from the action of the active Na extrusion mechanism, and does not require an additional consumption of metabolic energy supplies.

**Coupled NaCl Transport in Other Epithelia and the Effect of cAMP**

Shortly after Diamond postulated a neutral coupled transport mechanism to explain NaCl transport by fish gallbladder (1), evidence for a similar mechanism was presented for rabbit (2-4) and canine (36) gallbladders. In recent years, findings have been reported suggesting that this mechanism may be far more widely distributed throughout the alimentary canal. Tissues for which there is some evidence for coupled NaCl transport now include rabbit ileum (9, 10), human ileum (37), bullfrog small intestine (38), rat colon (39), rat jejunum (40, 41), guinea pig ileum (42), and bovine rumen (43).

Nellans et al. (9) have described a neutral, coupled NaCl influx process at the brush border of rabbit ileum which in all respects resembles that of rabbit gallbladder. In particular, Nellans et al. (9) demonstrated that the influx process in rabbit ileum is inhibited by agents that elevate intracellular cAMP concentrations and that this effect is at least in part responsible for the secretory state elicited by cholera enterotoxin. Cholera toxin has been shown to elevate intracellular cAMP levels in rabbit (44) gallbladder. Further, elevated cAMP levels in this tissue are associated with a marked inhibition of NaCl and fluid absorption and no change in the transepithelial PD (44). In canine gallbladder, in vivo, cholera toxin apparently elicits secretion (45). As shown in Table II, cAMP markedly inhibits $J_{Na}^{Na}$ and $J_{Cl}^{Cl}$ across rabbit gallbladder to the same extents; although we have not examined the effect of cAMP on Na and Cl influxes, on the basis of the data given in Table II and our previous studies on rabbit ileum it seems certain that the cAMP effect is attributable to an inhibition of $J_{NaCl}^{NaCl}$.

Several possible implications stem from these observations: First, rabbit gallbladder is comprised of a single cell type; these cells are responsible for the absorption of NaCl and water but are not capable of bringing about sugar or amino acid absorption. At least five different cell types have been identified in mammalian small intestine (46). Thus, it is quite possible that cells similar to those which comprise rabbit gallbladder are “sprinkled” throughout the intestinal tract, that these cells are responsible for coupled NaCl absorption alone, and that other cell types are responsible for Na-coupled sugar and amino acid absorption. It is of interest in this respect that rabbit gallbladder
cells do not possess the well-developed microvilli ("brush border") characteristic of the mature intestinal villus cells (47) that have been implicated in sugar and amino acid absorption (48).

Second, agents that elevate intracellular cAMP levels in rabbit ileum elicit active secretion of Cl and, in some instances, of Na (49). In rabbit gallbladder, an elevation of intracellular cAMP levels inhibits NaCl absorption but does not bring about secretion. Thus, it is possible that the overall response of rabbit ileum to elevated cAMP levels is the combined effect of an inhibition of coupled NaCl absorption by one cell type and the stimulation of secretion by another cell type.

Finally, as discussed by Schultz et al. (50), it is quite possible that under physiologic conditions the adenylcyclase-cAMP system in small intestine serves to regulate the rate at which ions and water are reabsorbed from the luminal contents. The finding that cAMP inhibits NaCl and water absorption by the gallbladder suggests that the rate at which bile is concentrated during interprandial periods may also be subject to humoral regulation.

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