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# The Mechanism of Anion Permeation in Thorium-Treated Gallbladder

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*Summary.* Polyvalent cations (such as $Ca^{+2}$, $La^{+3}$, and $Th^{+4}$) and low pH shift the permeability of some biological membranes from preferentially cation-permeable towards preferentially anion-permeable. The action of $Th^{+4}$ on permselectivity, and the anion permeation mechanism it produces, have been studied in rabbit gallbladder epithelium. The effect involves both a suppression of cation conductance and an increase of anion conductance. Most residual cation conductance is in a free-solution shunt. The anion permeability sequence, $SCN^- > ClO_4^- > NO_3^- > I^- > Br^- \geqq ClO_3^- > Cl^- > BrO_3^-$, is neither the lyotropic series nor a radius sequence but is in accord with considerations of equilibrium selectivity. Conductance increases linearly with bathing-solution salt concentrations from 10 mM up to at least 400 mM. The cation-to-anion permeability ratio shows little change with concentration up to at least 300 mM. The current-voltage relation is linear in symmetrical solutions and in the presence of a single-salt concentration gradient up to at least 570 mV. Dilution potentials and biionic potentials yield similar permeability ratios among anions. These permeability properties resemble (surprisingly) those of a membrane with fixed neutral sites rather than those of a classical ion-exchanger.

Rabbit gallbladder is preferentially permeable to cations at neutral pH, but becomes or approaches being preferentially permeable to anions at low pH or in the presence of polyvalent cations such as $Ca^{+2}$, $Ba^{+2}$, $La^{+3}$, and $Th^{+4}$ (Wright & Diamond, 1968). $H^+$ or polyvalent cations produce a similar permeability shift from cation-selective towards anion-selective in numerous other membranes, including barnacle muscle (Hagiwara, Gruener, Hayashi, Sakata & Grinnell, 1968), *Astacus* muscle (DeMello & Hutter, 1966), small intestine (Smyth & Wright, 1966), renal proximal tubule (Frömter & Lüer, 1969), choroid plexus (Wright & Prather, 1970), teeth (Amberson, Williams & Klein, 1926), lipid bilayer membranes (Gutknecht & Tosteson, 1970; McLaughlin, Szabo, Eisenman & Ciani, 1970), and artificial ion-exchange membranes (Helfferich, 1962).

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The present paper analyzes three problems in gallbladders rendered anion-selective by $Th^{+4}$: the mechanism of anion permeation; the selective permeability to different anions; and the basis of the permeability shift from cation-selective to anion-selective. We chose $Th^{+4}$ for detailed study because it produces a more marked and stable anion preference than do $H^+$, $Ca^{+2}$, and other polyvalent cations. However, the effects of all these agents on the gallbladder appear qualitatively similar. The high-conductance pathway for transepithelial ion permeation, and hence the site of the permeation mechanism discussed here, resides in the so-called tight junctions between the epithelial cells (Barry, Diamond & Wright, 1971; Diamond, Barry & Wright, 1971; Frömter & Diamond, 1972; Frömter, 1972). This paper parallels a series of four papers, which will be frequently cited in abbreviated form, on the mechanism of cation permeation in rabbit gallbladder: Barry and Diamond, 1970 and 1971, referred to as papers I and II, respectively; Wright, Barry and Diamond, 1971, cited as paper III; Barry, Diamond and Wright, 1971, cited as paper IV.

## Materials and Methods

Techniques for obtaining an in-vitro preparation of rabbit gallbladder and for measuring electrical potential differences (abbreviated p.d.'s), conductances, and current-voltage relations were essentially the same as those described previously (papers I, III and IV). In the conductance experiments the area of gallbladder used was 1.13 $cm^2$.

The gallbladder consists of a single uninterrupted layer of epithelial cells, held together at their free surface by membrane-fusion areas called tight junctions, and supported at the opposite surface by a layer of connective tissue. The bathing solution facing the epithelium and tight junctions is referred to as the mucosal solution, while the solution facing the connective tissue is referred to as the serosal solution. Since there is a thicker unstirred layer at the serosal surface of the epithelium than at the mucosal surface, dilution potentials and biionic potentials were generally measured by changing the ionic composition of the mucosal solution while holding the serosal solution constant. This procedure saves time without affecting the results obtained, since the permeability properties of the gallbladder are symmetrical (Diamond, 1962; Diamond & Harrison, 1966; papers III and IV; this paper, Fig. 9). When the composition of the mucosal and serosal solutions was changed symmetrically, a transient diffusion potential appeared because of the asymmetry of the unstirred layers, and we waited for this p.d. to decay before proceeding. All p.d.'s are reported as the potential of the mucosal solution with respect to that of the serosal solution (this is the opposite of the convention adopted in papers I–IV). All errors are given as standard errors of the mean.

The principal experimental solutions, all of which had an osmolarity of $292 \pm 7$ mOsm, were as follows: (a) The solution for use at neutral pH consisted of 150 mM NaCl, 0.25 mM $CaCl_2$, 0.375 mM $NaH_2PO_4$, and 2.125 mM $Na_2HPO_4$, with a pH of 7.35. (b) The standard solution for studying the effect of $Th^{+4}$ consisted of 150 mM NaCl or the $Na^+$ salt of another monovalent anion, plus 2.5 mM $Th(NO_3)_4$. The pH of these solutions was $3.02 \pm 0.15$ due to the formation of $Th(OH)_2^{+2}$ and $Th(OH)_3^+$ complexes

(Sillen & Martell, 1964), and no buffer other than $Th(NO_3)_4$ was necessary. All effects caused by $Th^{+4}$ discussed in this paper thus arise partly from an effect of low pH *per se* (p. 29, footnote 1). (c) A solution with composition 150 mM NaCl, 0.25 mM $CaCl_2$, buffered at pH 2.41 with 1.6 mM $K^+$-phthalate, was used in some experiments to achieve an anion-selective state at low pH without $Th^{+4}$. (d) Lower salt concentrations (for measuring dilution potentials or the conductance-concentration relation) were obtained by isosmotic replacement of salt with mannitol, based on an osmotic coefficient of 1.00 for mannitol and osmotic coefficients for salts taken from Robinson & Stokes (1965) or Parsons (1959). In the presence of $Th(NO_3)_4$ isosmotic replacement of salt with mannitol was found to lower the pH by 0.5 pH units, and the pH was readjusted to 3.02 with 1 N NaOH. All solutions were prepared on a molal basis (moles solute per kg water, not per liter solution), since the fractional solution volumes occupied by solutes were not negligible.

Experiments were performed at 22 to 27 °C, the variation within any experiment being less than 1 °C.

P.d.'s were recorded with calomel half-cells connected to the bathing solutions by polyethylene bridges filled with appropriate salt solutions immobilized by 5% agar. Accurate correction of measured p.d.'s for junction potentials was essential in the present study, since junction potentials with the solutions used were frequently up to 4 mV and occasionally up to 9 mV, while the experimental p.d.'s were always less than 25 mV and frequently less than 10 mV. To obtain stable, well-defined, calculable junction potentials, the salt composition of the bridges was chosen so that all junctions were of either the so-called dilution junction type or the so-called biionic junction type. For measurement of dilution potentials (the p.d. resulting from $Na^+X^-$ ($C_1$) *vs.* $Na^+X^-$ ($C_2$), where $C_1$ was generally set at 150 mM and $C_2$ at 75 mM), the salt bridges contained the given salt $Na^+X^-$ at 150 mM. For measurement of biionic potentials (the p.d. resulting from $Na^+X^-$ ($C_1$) *vs.* $Na^+Y^-$ ($C_1$), where $C_1$ was always set at 150 mM, $X^-$ was always $Cl^-$ or $NO_3^-$, and $Y^-$ was any other anion), the salt bridges contained $Na^+X^-$ (i.e., NaCl or $NaNO_3$) at 150 mM. The method of calculating values of the resulting junction potentials, which were subtracted from all measured p.d.'s, is given in the legend to Table 1, and the values are listed in Table 1. The problem of junction potentials is discussed in detail in paper I.

In most experiments the mucosal and serosal bathing solutions had the same pH and [$Th^{+4}$], but the experiments described on pp. 68–69 involved $Th^{+4}$ or low pH on only one side of the gallbladder. To correct NaCl dilution potentials measured under these conditions of asymmetrical pH for the resulting $H^+$ diffusion potential (generally several mV in magnitude), the p.d. with the same asymmetrical pH or [$Th^{+4}$] but with symmetrical [NaCl] was measured separately and subtracted from the NaCl dilution potential.

Relative permeability coefficients were extracted from measured p.d.'s by the same model equations and procedures used previously in analyzing cation permeation (papers II and IV), except that the equations were reexpressed in terms of two anions and one cation rather than two cations and one anion. Specifically, the equations used were the Goldman-Hodgkin-Katz or "constant-field" equation [paper IV, Eq. (1)]; the equations describing a thick membrane with fixed neutral sites [paper II, Eqs. (42) and (43)]; the equations describing a thick membrane with fixed neutral sites and a shunt, assuming cation mobility in the neutral-site channel to be zero [paper II, Eq. (53)]; and the equations describing a thin membrane with fixed neutral sites and a shunt, assuming cation mobility in the neutral-site channel to be zero [paper II, Eqs. (110) and (113)]. $Ca^{+2}$ and $Th^{+4}$ were considered impermeant (*see* paper III). The small amount (10 mM) of $NO_3^-$ added as $Th(NO_3)_4$ is not impermeant, and its presence in solutions whose principal salt is not $NaNO_3$ introduces a minor complication, since the model equations, other

Table 1. Calculated free-solution junction potentials

| Solution 1 | Solution 2 | Calculated p.d. (mV) |
|---|---|---|
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaBr + 2.5 $Th(NO_3)_4$ | 0.3 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | −1.2 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaI + 2.5 $Th(NO_3)_4$ | 0.2 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 $NaClO_4$ + 2.5 $Th(NO_3)_4$ | −1.9 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaSCN + 2.5 $Th(NO_3)_4$ | −2.1 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 $NaClO_3$ + 2.5 $Th(NO_3)_4$ | −2.6 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 150 $NaBrO_3$ + 2.5 $Th(NO_3)_4$ | −4.5 |
| 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 150 NaBr + 2.5 $Th(NO_3)_4$ | 1.6 |
| 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 150 NaI + 2.5 $Th(NO_3)_4$ | 1.4 |
| 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 150 $NaClO_4$ + 2.5 $Th(NO_3)_4$ | −0.7 |
| 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 150 NaSCN + 2.5 $Th(NO_3)_4$ | −0.9 |
| 75 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaCl + 2.5 $Th(NO_3)_4$ | 3.4 |
| 75 NaBr + 2.5 $Th(NO_3)_4$ | 150 NaBr + 2.5 $Th(NO_3)_4$ | 3.5 |
| 75 NaI + 2.5 $Th(NO_3)_4$ | 150 NaI + 2.5 $Th(NO_3)_4$ | 3.4 |
| 75 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 150 $NaNO_3$ + 2.5 $Th(NO_3)_4$ | 2.7 |
| 75 $NaClO_4$ + 2.5 $Th(NO_3)_4$ | 150 $NaClO_4$ + 2.5 $Th(NO_3)_4$ | 2.4 |
| 75 NaSCN + 2.5 $Th(NO_3)_4$ | 150 NaSCN + 2.5 $Th(NO_3)_4$ | 2.3 |
| 37.5 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaCl + 2.5 $Th(NO_3)_4$ | 6.4 |
| 18.75 NaCl + 2.5 $Th(NO_3)_4$ | 150 NaCl + 2.5 $Th(NO_3)_4$ | 9.2 |
| 150 NaCl + 0.25 $CaCl_2$ + 0.375 $NaH_2PO_4$ + 2.125 $Na_2HPO_4$ | 75 NaCl + 0.25 $CaCl_2$ + 0.375 $NaH_2PO_4$ + 2.125 $Na_2HPO_4$ | −3.7 |
| 150 NaCl + 0.25 $CaCl_2$ + 1.6 $K^+$ phthalate | 75 NaCl + 0.25 $CaCl_2$ + 1.6 $K^+$ phthalate | −3.7 |
| 150 NaCl + 2.5 $Th(NO_3)_4$ | 75 NaCl + 0.25 $CaCl_2$ + 0.375 $NaH_2PO_4$ + 2.125 $Na_2HPO_4$ | −4.2 |
| 150 NaCl + 0.25 $CaCl_2$ + 0.375 $NaH_2PO_4$ + 2.125 $Na_2HPO_4$ | 75 NaCl + 2.5 $Th(NO_3)_4$ | −3.2 |

Junction potentials at 24 °C were calculated from a modified Planck-Henderson equation [paper I, pp. 102–103 and 118–121, Eqs. (1) and (2)]. The third column gives the potential of the solution listed in column 2, with respect to the solution listed in column 1. Solution composition is given in mM. In the last two entries, where there is a pH gradient, its contribution to the junction potential was also taken into account.

In obtaining single-ion activity coefficients ($\gamma$'s) and mobilities ($u$'s) to substitute into Eqs. (1) and (2) of paper I, the following assumptions were made: (a) $\gamma$'s were obtained from Robinson and Stokes (1965), using the Guggenheim assumption that single-ion activity coefficients equal mean activity coefficients. (b) Ionic mobilities are somewhat dependent on concentration. In NaCl solutions, $u$'s for $Na^+$ and $Cl^-$ at the actual salt concentration used were taken from Longsworth (1932). For other $Na^+$

than the Goldman-Hodgkin-Katz equation, become excessively complex if one incorporates terms for three rather than two anions or if the concentrations of the principal anion and principal cation in a given solution are not identical. The approximation used was to treat the small amount of $NO_3^-$ as equivalent to the principal anion present and to average the concentrations of the principal anion and principal cation—i.e., to add 5 mM to their actual values and to omit $NO_3^-$ terms. Activity coefficients ($\gamma$'s) were estimated as discussed in the legend to Table 1. All calculations were carried out on an IBM 360 computer, using APL language.

Immediately after dissection, the dilution potential resulting from a 150:75 mM NaCl gradient at pH 7.35 was measured, and gallbladders yielding a value less than 8.5 mV were rejected. The properties of $Th^{+4}$-treated gallbladders gradually deteriorate with time as if a free-solution shunt were developing (pp. 71–76), and dilution potentials and biionic potentials decay towards free-solution junction potentials. The deterioration is more rapid than that observed at neutral pH (papers III and IV). However, it is slower than that observed at pH 2.4 in the absence of $Th^{+4}$, indicating that $Th^{+4}$ partially protects the gallbladder against the deleterious effects of low pH, and this fact provided the main reason for using $Th^{+4}$ in this study of anion permeation. Because of this gradual deterioration all experiments involved a "bracketed" design, in which each experimental measurement was immediately preceded and followed by some standard measurement (e.g., conductance in 150 mM NaCl, or else the 150:75 mM NaCl dilution potential). Thus, measurements could either all be interpolated to the same time for comparison, or else be expressed as a fraction of a standard measurement. Figs. 4, 7, and 9 illustrate examples of this bracketed design.

## Results

This Results section considers in turn three problems: the changes which $Th^{+4}$ produces in the ion permeability of the gallbladder; anion selectivity in $Th^{+4}$-treated gallbladders; and the anion permeation mechanism in $Th^{+4}$-treated gallbladders.

### *Effects of $Th^{+4}$*

*Effects on p.d.'s.* At pH 7.35, gallbladders were found to be much more permeable to cations than to anions, as analyzed in detail previously (Dia-

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salts for which less complete experimental information concerning the concentration dependence of $u$'s is available, $u$'s at the required concentration were calculated from $u$'s at infinite dilution, assuming the concentration dependence of the total salt conductivity and of the partial anion conductivity to be the same as that for NaCl and $Cl^-$ in NaCl solutions (Robinson & Stokes, 1965). This assumption is justified by the limited available information (Parsons, 1959). (c) 2.5 mM $Th(NO_3)_4$ was assumed to have the same effect in depressing $u$'s and $\gamma$'s of the principal salt present as would an additional 10 mM of that salt. (d) Since the equations apply to monovalent ions, 2.5 mM $Th^{+4}$ was considered equivalent to 10 mM of a monovalent cation with a conductivity of 27.8 $cm^2$/ohm-equivalent, the value for $Th^{+4}$ at infinite dilution (Parsons, 1959). (e) $u$ for $NO_3^-$, added as $Th(NO_3)_4$ to solutions of other salts, was taken as the value at a $NO_3^-$ concentration equal to the total anion concentration of the solution.

mond, 1962; Diamond & Harrison, 1966; Wright & Diamond, 1968; papers III and IV). The average p.d. resulting from a 2:1 concentration gradient of NaCl (serosal solution 150 mM, mucosal solution 75 mM) was $11.10 \pm 0.45$ mV in three gallbladders (i.e., mucosal solution positive to the more concentrated serosal solution). This yields a value of $P_{Cl}/P_{Na} = 0.16 \pm 0.02$ calculated from the Goldman-Hodgkin-Katz equation. Biionic potentials resulting from cation gradients (e.g., 150 mM NaCl *vs.* 150 mM KCl) were up to 22 mV, while biionic potentials resulting from anion gradients (e.g., 150 mM NaCl *vs.* 150 mM NaBr, $NaNO_3$, NaI, $NaClO_4$, or NaSCN) were generally less than 0.75 mV. Most of this small anion conductance at neutral pH is in a free-solution shunt rather than in native membrane (paper IV).

When 2.5 mM $Th^{+4}$ was added to both the mucosal and serosal bathing solutions, the sign of the 2:1 NaCl dilution potential reversed (mucosal solution negative to the more concentrated serosal solution), indicating that the epithelium had now become selectively permeable to anions. The effect became maximal at 7 to 15 min after exposure, when the 2:1 NaCl dilution potential reached $-11.13 \pm 0.26$ mV ($n = 11$), yielding a calculated $P_{Na}/P_{Cl} = 0.13 \pm 0.01$, while the p.d. at 6 min or less was $-9.49 \pm 0.18$ mV ($n = 4$) ($P_{Na}/P_{Cl} = 0.22 \pm 0.01$). After 15 min, the p.d. slowly began to decline (*see* Fig. 1). Other polyvalent cations such as $Ca^{+2}$, $Ba^{+2}$, $La^{+3}$, and $UO_2^{+2}$ produce a qualitatively similar but smaller effect on dilution potentials, such that the initially cation-selective p.d. decreases in magnitude but does not reverse in sign (Wright & Diamond, 1968). The effect of $Th^{+4}$-containing solutions is probably due in part to their low pH (3.02), but there must also be a considerable effect unrelated to pH, since pH 3 solutions without $Th^{+4}$ reduce dilution potentials to near zero but fail to reverse their sign. A further expression of the anion selectivity produced by $Th^{+4}$ is that anion gradients now yield large biionic potentials (pp. 73–76).

The effect of $Th^{+4}$ added to the serosal solution alone was considerably smaller and slower to develop. At the end of 2 hr the 2:1 NaCl dilution potential was still $4.75 \pm 0.35$ mV in two experiments, the sign indicating continued cation preference ($P_{Cl}/P_{Na} = 0.34 \pm 0.03$), although addition of $Th^{+4}$ after 2 hr to the mucosal solution as well yielded an anion-selective dilution potential in the same two gallbladders ($-10.35 \pm 0.25$ mV, $P_{Na}/P_{Cl} = 0.17 \pm 0.01$). Similarly, in one further experiment the effect of low pH in the absence of $Th^{+4}$ was smaller and slower from the serosal side alone than from both sides simultaneously. With the mucosal solution maintained at pH 7.35, a serosal solution with pH 2.4 still left the gallbladder slightly

cation-selective after 30 min (2:1 NaCl dilution potential = 5.55 mV, $P_{Cl}/P_{Na} = 0.45$) and after 1 hr, although briefly changing the mucosal solution to pH 2.4 as well yielded a strongly anion-selective dilution potential ($-7.95$ mV, $P_{Na}/P_{Cl} = 0.32 \pm 0.01$).

The effect of $Th^{+4}$ added to the mucosal solution alone was almost as large, and developed as rapidly, as the effect produced by $Th^{+4}$ in both solutions simultaneously. The sign of NaCl dilution potentials reversed within 15 sec after the start of exposure to mucosal $Th^{+4}$ and went increasingly negative during the next several minutes, with a time course similar to that of the effect of low mucosal pH in the absence of $Th^{+4}$. In two gallbladders exposed to $Th^{+4}$ in the mucosal solution the average maximal 2:1 NaCl dilution potential was $-7.88 \pm 0.89$ mV ($P_{Na}/P_{Cl} = 0.23 \pm 0.05$). The 2:1 NaCl dilution potential in the same two gallbladders when $Th^{+4}$ was added to both the mucosal and serosal solutions was only slightly larger, $-10.09 \pm 0.75$ mV ($P_{Na}/P_{Cl} = 0.19 \pm 0.04$). Anion biionic potentials were as large (up to 25 mV) with $Th^{+4}$ in the mucosal solution alone as with $Th^{+4}$ in both bathing solutions. Addition of $Th^{+4}$ to the mucosal solution was also found to reverse the sign of apparent streaming potentials, i.e., the p.d. produced by addition of an impermeant nonelectrolyte to one bathing solution in the absence of ion concentration gradients (Diamond & Harrison, 1966; Wedner & Diamond, 1969). The hypertonic solution became positive in the untreated gallbladder, negative after $Th^{+4}$ treatment, again indicating a reversal of permselectivity.

When the mucosal surface was exposed to $Th^{+4}$ for less than 10 min, its effect on ion permeability as judged by dilution potentials was almost completely reversible. However, after exposure of the mucosal surface alone or else both surfaces to $Th^{+4}$ for 50 to 60 min, repeated washing with $Th^{+4}$-free solutions for up to 1 hr reduced anion-selective dilution potentials to less than the free-solution junction potential but did not restore cation-selective dilution potentials. Since these washed gallbladders still gave large anion-selective p.d.'s on reexposure to $Th^{+4}$, the poor reversibility of the $Th^{+4}$ effect suggests $Th^{+4}$ binding to the membrane and cannot be attributed to non-specific damage or the opening of a shunt. $La^{+3}$ at 1 mM exerts similar but smaller effects on dilution potentials, and, like the $Th^{+4}$ effect, the $La^{+3}$ effect is completely reversible after short exposure times (Wright & Diamond, 1968) but only poorly reversible after 60 min (Machen, Erlij & Wooding, 1972).

Thus, $Th^{+4}$-treated gallbladders yield anion-selective diffusion potentials as large as the cation-selective potentials observed before treatment. The

Table 2. Changes in gallbladder conductance due to $Th^{+4}$

| Solution | Conductance (mmho) |
|---|---|
| NaCl, pH 7 | 34.2±1.1 |
| NaCl, $Th^{+4}$ (30 sec) | 17.5±2.0 |
| NaCl, $Th^{+4}$ (10 min) | 44.2±9.3 |
| NaCl, pH 7 | 44.8±0.2 |
| choline chloride, pH 7 | 14.6±0.1 |
| choline chloride, $Th^{+4}$ (30 sec) | 16.6±0.1 |
| choline chloride, $Th^{+4}$ (10 min) | 60.5±9.5 |

In the first set of experiments (first three rows), the conductance of each of six gallbladders was measured in 150 mM NaCl solutions, first at pH 7 and then at 30 sec and 10 min after addition of 2.5 mM $Th(NO_3)_4$ to both bathing solutions. Note that conductance first decreases, then increases, after exposure to $Th^{+4}$. In the second set of experiments (last four rows), the conductance of each of two gallbladders was measured in 150 mM NaCl at pH 7, then in 150 mM choline chloride, first at pH 7 and then 30 sec and 10 min after addition of 2.5 mM $Th(NO_3)_4$ to both bathing solutions. Note that conductance in choline chloride is lower than in NaCl, and increases immediately after exposure to $Th^{+4}$ without any initial decrease.

effect is mainly exerted from the mucosal surface, and is reversible after short times but only partially reversible after long times.

*Immediate Effect on Conductance.* The inversion in $P_{Cl}/P_{Na}$ produced by $Th^{+4}$ might be due either to a decrease in cation conductance ($G_{Na}$), an increase in anion conductance ($G_{Cl}$), or both. This problem was investigated by measuring the effect of $Th^{+4}$ on gallbladder conductance (Table 2).

When the gallbladder was initially bathed in 150 mM NaCl Ringer's solution at pH 7.35 and $Th^{+4}$ was added to both bathing solutions, there was an immediate (i.e., within 30 sec) decrease in conductance to 51% of the initial value (compare first two rows, Table 2). This decrease was followed by a slower increase (compare second and third row, Table 2), whose origin is discussed on pp. 71–76. The initial decrease is probably due at least in part to a decrease in $G_{Na}$, but the following calculation shows that this cannot be the sole change occurring. In untreated gallbladders the average value of $P_{Cl}/P_{Na}$ is 0.16, so that $G_{Na}$ represents $(100)(1.00)/(1.00+0.16)=86\%$ of the total conductance. If the $Th^{+4}$-induced decrease in total conductance to 51% of the initial value were solely at the expense of $G_{Na}$, $G_{Na}$ would decrease to $(100)[1.0-(0.49)(1.16)]=43\%$ of its initial value,

and $P_{Cl}/P_{Na}$ would increase only to $0.16/0.43 = 0.37$. In fact, $P_{Cl}/P_{Na}$ is already greater than 1.0 after 30-sec exposure to $Th^{+4}$. Hence, the 49% initial decrease in total conductance must consist of a still larger decrease in $G_{Na}$, partially masked by an increase in $G_{Cl}$.

This interpretation is consistent with the conductance changes observed in choline chloride Ringer's solution. Since choline is much less permeant than $Na^+$ (Wright & Diamond, 1968), at pH 7.35 the total conductance is 67% lower in choline chloride than in NaCl (compare lines 4 and 5, Table 2), and $G_{Cl}$ accounts for a much greater fraction of the total conductance. The immediate effect of $Th^{+4}$ in choline chloride is a conductance increase (compare lines 5 and 6, Table 2), presumably the expected increase in $G_{Cl}$. The further increase in total conductance from 30 sec to 10 min in either NaCl or choline chloride (compare lines 2 and 3, or 6 and 7, Table 2) probably represents a continued increase in $G_{Cl}$, with a possible contribution from the opening of a shunt (*see* next section).

Thus, the inversion in $P_{Cl}/P_{Na}$ caused by $Th^{+4}$ involves both a decrease in $G_{Na}$ and an increase in $G_{Cl}$. The same conclusion applies to the inversion in $P_{Cl}/P_{Na}$ caused by $H^+$, both in gallbladder (Wright & Diamond, 1968) and in barnacle muscle (Hagiwara *et al.*, 1968).

*The Shunt.* After the first 7 min of exposure to $Th^{+4}$, during which there are rapid conductance changes associated with a rapid inversion in $P_{Cl}/P_{Na}$, $G$ slowly increases throughout the remainder of the experiment, associated with a slow shift in $P_{Cl}/P_{Na}$ back towards the free-solution mobility ratio. This slow phase is very similar to the slow changes in $G$ and in $P_{Cl}/P_{Na}$ which occur in cation-selective gallbladders maintained at neutral pH and which are due to the development of a free-solution shunt, perhaps as a result of gradual deterioration of the tissue (paper IV). The origin of the slow changes in $Th^{+4}$-treated gallbladders was investigated in the experiment depicted in Fig. 1, in which conductance and NaCl dilution potentials were both measured as a function of time in the same gallbladder. From the initial value of the NaCl dilution potential and of the conductance at this time, 26 min after $Th^{+4}$ had been added to both bathing solutions, subsequent values of the dilution potential were calculated on the basis of each of three hypotheses concerning the origin of the slow conductance increase: that it represents (a) solely an increase in $G_{Cl}$, (b) solely an increase in $G_{Na}$, (c) the development of a free-solution shunt, i.e., an increase in both $G_{Na}$ and $G_{Cl}$ in the ratio of their free-solution mobilities. It is clear from Fig. 1 and from the same experiment in another gallbladder that the shunt hypothesis gives the closest fit.

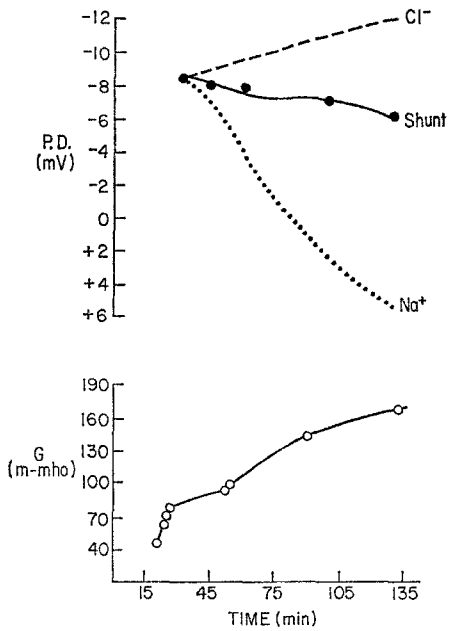


Fig. 1. Comparison of 2:1 NaCl dilution potentials (•) and of conductance (◦) in symmetrical 150 mM NaCl solutions, measured as a function of time after addition of 2.5 mM $Th(NO_3)_4$ to both bathing solutions. The ratio $P_{Na}/P_{Cl}$ was calculated from the earliest p.d. measurement (−8.5 mV at 26 min) by means of the Goldman-Hodgkin-Katz equation to be 0.22. The total membrane conductance $G$ at 26 min, 46.2 mmho, must therefore be assigned to partial conductances $G_{Na}$ and $G_{Cl}$ in this ratio of 0.22, after multiplication of $G$ by 150/160 to account for the 10 mM $[NO_3^-]$ present in the bathing solution in addition to 150 mM NaCl: i.e., $G_{Na}=\left(\frac{0.22}{1.00+0.22}\right)\left(\frac{150}{160}\right)(46.2)=7.9$ mmho, $G_{Cl}=\left(\frac{1.00}{1.00+0.22}\right)\left(\frac{150}{160}\right)(46.2)=38.3$ mmho. The subsequent course of the p.d. with time was then calculated from the subsequent increase in conductance $\Delta G$ above 46.2 mmho, according to each of three different assumptions: (1) $\Delta G$ represents solely an increase in $G_{Cl}$, so that $P_{Na}/P_{Cl}$ at any time should equal $(7.9)/(38.3+\Delta G)$. These calculated values of $P_{Na}/P_{Cl}$ were inserted into the Goldman-Hodgkin-Katz equation to yield the predicted p.d. curve -------. (2) $\Delta G$ represents solely an increase in $G_{Na}$, so that $P_{Na}/P_{Cl}$ at any time should equal $(7.9+\Delta G)/38.3$, yielding the predicted curve ..... (3) $\Delta G$ represents the opening of a free-solution shunt (with transport numbers $t_{Cl}=0.62$, $t_{Na}=0.38$), so that $P_{Cl}/P_{Na}$ at any time should equal $(7.9+0.38\ \Delta G)/(38.3+0.62\ \Delta G)$, yielding the predicted curve ——. Note that the experimental p.d.'s fit the third assumption

In cation-selective gallbladders at neutral pH virtually all of the low chloride conductance is in the shunt rather than in native membrane (paper IV). The following experiment was therefore designed to examine whether the shunt might similarly account for almost all the low sodium conductance in gallbladders rendered anion-selective with $Th^{+4}$. The

principle of the experiment was to compare the calculated shunt $G_{Na}$ in gallbladders at neutral pH with the total $G_{Na}$ after treatment with $Th^{+4}$, by measuring conductance and dilution potentials in three gallbladders in 150 mM NaCl Ringer's solution immediately before and after addition of $Th^{+4}$ to both bathing solutions. At neutral pH, immediately before addition of $Th^{+4}$, the conductance of these three gallbladders was $33.8 \pm 2.1$ mmho, and $P_{Cl}/P_{Na}$ extracted from dilution potentials was $0.16 \pm 0.03$. $G_{Cl}$ was therefore $(33.8)(0.16/1.16) = 4.7$ mmho. Since essentially all this $G_{Cl}$ was in a shunt in which ion conductances are in the free-solution mobility ratios, and since $u_{Na}/u_{Cl}$ in 150 mM NaCl is 0.62, the shunt must have contained a $G_{Na}$ of $(0.62)(4.7) = 2.9$ mmho. In the cation-selective state this is only a small fraction of the total $G_{Na}$ ($33.8 - 4.7 = 29.1$ mmho), most of which is in native membrane. Immediately (30 sec) after addition of $Th^{+4}$ the total conductance in these three gallbladders was $22.2 \pm 2.1$ mmho, or 21.8 mmho after subtraction of the small conductance associated with the 10 mM $NO_3^-$ added as $Th(NO_3)_4$. $P_{Na}/P_{Cl}$ extracted from dilution potentials was $0.17 \pm 0.01$. Thus, the total $G_{Na}$ in these $Th^{+4}$-treated gallbladders was $(21.8)(0.17/1.17) = 3.2$ mmho. Since the $G_{Na}$ that was already present in the shunt was virtually the same, 2.9 mmho, $G_{Na}$ in native membrane after $Th^{+4}$ treatment must be negligible. Virtually all of the low sodium conductance remaining in gallbladders treated with $Th^{+4}$ is therefore in the shunt.

### *Anion Selectivity in $Th^{+4}$-Treated Gallbladders*

In this section we consider the relative permeability coefficients ($P$'s) of the gallbladder for different anions after $Th^{+4}$ treatment.

In principle, anion permeabilities could be compared in three different ways: from measurements of conductances, dilution potentials, or biionic potentials. Biionic potentials require changing only the mucosal solution and can be measured quickly, while comparison of dilution potentials or conductances requires changing both the serosal and mucosal solutions and is more time-consuming because of equilibration delays in the connective tissue at the serosal surface of the epithelium. This section presents the biionic potential measurements, which will be compared with dilution potential measurements on p. 85. The experimental procedure was to maintain the serosal solution constant as either 150 mM NaCl or 150 mM $NaNO_3$, and to measure the biionic potentials produced by repeatedly changing the mucosal solution in succession to 150 mM NaBr, NaI, $NaClO_4$, NaSCN, $NaClO_3$, and $NaNO_3$ or NaCl. In addition, the mucosal solution

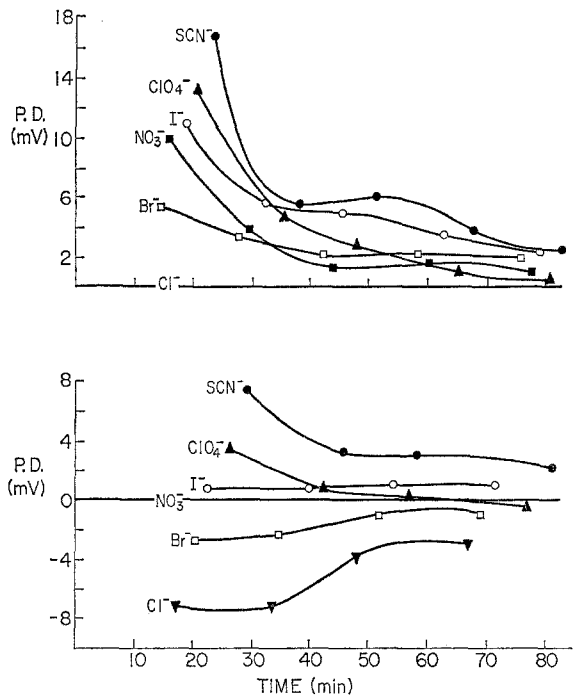


Fig. 2. Biionic potentials across the gallbladder (ordinate), as a function of time after addition of 2.5 mM $Th(NO_3)_4$ to the mucosal and serosal solutions. Throughout the experiment the serosal solution contained 150 mM NaCl (upper half of figure) or 150 mM $NaNO_3$ (lower half of figure). The mucosal solution was sequentially changed to 150 mM NaSCN (•), $NaClO_4$ (▲), NaI (○), $NaNO_3$ (■), NaBr (□), and NaCl (▼). A positive p.d. means that the given anion is more permeant than $Cl^-$ (upper half of figure) or than $NO_3^-$ (lower half). Biionic potentials decline with time. However, cation-anion discrimination shows little decline: the 2:1 NaCl dilution potential was still −10.50 mV ($P_{Na}/P_{Cl}=0.16$) after 55 min in the upper experiment, and the 2:1 $NaNO_3$ dilution potential was −10.25 mV ($P_{Na}/P_{NO_3}=0.16$) after 65 min in the lower experiment

was periodically changed to 75 mM NaCl or $NaNO_3$ in order to record the 2:1 dilution potential. All solutions contained 2.5 mM $Th(NO_3)_4$.

The upper half of Fig. 2 illustrates the results of an experiment in which the serosal solution was 150 mM NaCl, while the serosal solution was 150 mM $NaNO_3$ in the experiment depicted in the lower half of Fig. 2. In both cases, the permeability sequence is $SCN^- > ClO_4^- > I^- > NO_3^- > Br^- > Cl^-$ soon after dissection. During the course of 1 hr, the biionic potentials decrease towards small values, with reversals in the relative positions of several ions. NaCl and $NaNO_3$ dilution potentials also decrease slightly with time (*cf.* Fig. 1), but by much less than do biionic potentials. After 1 hr, dilution potentials still yield cation-to-anion permeability ratios far removed from

Table 3. Relative anion permeabilities from biionic potentials in $Th^{+4}$-treated gallbladders

| Anion | Relative permeability | |
|---|---|---|
| | From biionics *vs.* NaCl | From biionics *vs.* $NaNO_3$ |
| $SCN^-$ | $1.98 \pm 0.12$ (8) | $2.35 \pm 0.28$ (3) |
| $ClO_4^-$ | $1.50 \pm 0.06$ (14) | $1.76 \pm 0.05$ (4) |
| $NO_3^-$ | $1.43 \pm 0.05$ (12) | $1.57 \pm 0.03$ (4) |
| $I^-$ | $1.44 \pm 0.05$ (10) | $1.53 \pm 0.01$ (3) |
| $Br^-$ | $1.21 \pm 0.02$ (12) | $1.24 \pm 0.04$ (3) |
| $ClO_3^-$ | $1.19 \pm 0.02$ (6) | – |
| $Cl^-$ | 1.00 | 1.00 |
| $BrO_3^-$ | $0.83 \pm 0.00$ (3) | – |

Biionic potentials were measured against 150 mM NaCl or 150 mM $NaNO_3$ 25 min after exposure to 2.5 mM $Th(NO_3)_4$, as illustrated in Fig. 2. Permeability coefficients relative to $P_{Cl} = 1.0$ were calculated from the equation for a thin fixed-neutral-site membrane plus shunt [paper II, Eq. (113)]. When biionic potentials were measured against $NaNO_3$, the permeability coefficient relative to $NO_3^-$ was first calculated and then multiplied by the value of $P_{NO_3}/P_{Cl}$ determined in the same experiment to obtain the permeability coefficient relative to $Cl^-$. Numbers in parentheses are the numbers of different gallbladders used. Note that comparison with either NaCl or $NaNO_3$ yields essentially the same permeability sequence and similar ratios, $SCN^- > ClO_4^- > NO_3^- \sim I^- > Br^- \geqq ClO_3^- > Cl^- > BrO_3^-$.

free-solution mobility ratios (average $P_{Na}/P_{Cl} = 0.30 \pm 0.02$ (n = 26) from NaCl dilution potentials, average $P_{Na}/P_{NO_3} = 0.19 \pm 0.01$ (n = 4) from $NaNO_3$ dilution potentials). Thus, with time, the epithelium loses much of its ability to discriminate between different anions but retains its ability to select anions over cations.

*P*'s were calculated from biionic potentials interpolated to 25 min after dissection, using the equation describing a membrane with fixed neutral sites and a shunt [paper II, Eq. (113)]. As seen in Table 3, the whole permeability sequence at 25 min is $SCN^- > ClO_4^- > NO_3^- \sim I^- > Br^- \geqq ClO_3^- > Cl^- > BrO_3^-$. The order is essentially the same, and the values similar, whether biionic potentials are measured against NaCl or against $NaNO_3$.

In six experiments, permeability properties were studied systematically as a function of time after dissection for at least 1 hr, by maintaining the serosal solution as 150 mM NaCl while changing the mucosal solution repeatedly in turn to 150 mM NaSCN, 150 mM $NaClO_4$, 150 mM NaI, 150 mM $NaNO_3$, 150 mM NaBr, and 75 mM NaCl. Table 4 gives *P*'s extracted from biionic potentials interpolated to 20, 30 and 60 min after dissection, using either the fixed-neutral-site-plus-shunt equation or the

Table 4. Relative anion permeabilities as a

| Time (min) | $P_{SCN}/P_{Cl}$ | | $P_{ClO_4}/P_{Cl}$ | |
|---|---|---|---|---|
| | GHK | TNS | GHK | TNS |
| 20 | 1.86 ±0.31 (5) | 2.22 ±0.46 (5) | 1.61 ±0.23 (3) | 1.89 ±0.34 (3) |
| 30 | 1.37 ±0.09 (5) | 1.64 ±0.13 (5) | 1.22 ±0.07 (4) | 1.40 ±0.10 (4) |
| 60 | 1.06 ±0.04 (2) | 1.22 ±0.06 (2) | 1.00 ±0.02 (3) | 1.10 ±0.02 (3) |
| Free-solution *u* ratio | 0.86 | | 0.88 | |

Biionic potentials against NaCl were measured in six gallbladders at various times after exposure to 2.5 mM $Th(NO_3)_4$, as illustrated in Fig. 2. From p.d.'s interpolated at 20, 30, and 60 min, permeability coefficients relative to $P_{Cl}=1.0$ were calculated from the Goldman-Hodgkin-Katz equation ["GHK": paper IV, Eq. (1)] or from the equation for a thin fixed-neutral-site membrane plus shunt ["TNS": paper II, Eq. (113)].

Goldman-Hodgkin-Katz equation. The two sets of values are qualitatively similar, but the Goldman-Hodgkin-Katz values are smaller, because they do not correct for the effect of the shunt. Even after correction for the effect of the shunt by the fixed-neutral-site-plus-shunt equation, however, $P$'s decrease with time towards the free-solution mobility ratios: i.e., anion – anion discrimination as evidenced by biionic potentials declines more rapidly than does anion – cation discrimination as evidenced by dilution potentials. There is a similar but less marked time dependence in cation-selective gallbladders (paper IV, Table 5), suggesting some change in the properties of the permeation channel with time after dissection.

The earliest sequence that could be determined, the one at 20 min after dissection (Table 4), differs from the 25-min sequence only in that $NO_3^-$ is more permeant than $I^-$ instead of comparable to $I^-$. The whole sequence at 20 min is therefore $SCN^- > ClO_4^- > NO_3^- > I^- > Br^- \geqq ClO_3^- > Cl^- > BrO_3^-$. This is neither the sequence of the free-solution mobilities nor the sequence of calculated so-called "hydrated radii" nor the sequence of ionic radii.

### *The Anion Permeation Mechanism in $Th^{+4}$-Treated Gallbladders*

This final section of the Results analyzes the anion permeation mechanism in $Th^{+4}$-treated gallbladders by the same approach used previously to study the cation permeation mechanism at neutral pH (papers III and IV).

function of time in $Th^{+4}$-treated gallbladders

| $P_I/P_{Cl}$ | | $P_{NO_3}/P_{Cl}$ | | $P_{Br}/P_{Cl}$ | |
|---|---|---|---|---|---|
| GHK | TNS | GHK | TNS | GHK | TNS |
| 1.30 (1) | 1.44 (1) | 1.46 (1) | 1.81 (1) | 1.28 ±0.03 (2) | 1.36 ±0.01 (2) |
| 1.23 ±0.03 (4) | 1.33 ±0.03 (4) | 1.23 ±0.33 (5) | 1.37 ±0.04 (5) | 1.13 ±0.03 (3) | 1.19 ±0.03 (3) |
| 1.09 (1) | 1.16 (1) | 1.06 ±0.04 (3) | 1.16 ±0.06 (3) | 1.06 ±0.01 (2) | 1.10 ±0.02 (2) |
| 1.01 | | 0.93 | | 1.02 | |

Numbers in parentheses are numbers of measurements. The last row gives the relative mobilities in free solution. Note that relative permeabilities approach relative free-solution mobilities with time, even after correction for the effect of the shunt (i.e., "TNS" values are further removed from free-solution mobility ratios than are "GHK" values but still show time dependence).

Briefly, the approach is to determine "black-box" characteristics of ion permeation and to compare these with the "black-box" characteristics of artificial membranes having a well-understood structure and permeation mechanism. Five characteristics were studied: the conductance–concentration relation, the concentration dependence of cation-to-anion discrimination, the current–voltage relation, dilution potentials as a function of gradient, and comparison of dilution potentials and biionic potentials.

*The Conductance-Concentration Relation.* Conductance was measured in symmetrical solutions of NaCl or $NaNO_3$ in the presence of 2.5 mM $Th(NO_3)_4$, varying the NaCl concentration from 0 to 400 mM or the $NaNO_3$ concentration from 25 to 400 mM while maintaining osmolarity constant with mannitol. Since conductance in any given bathing solution gradually increases with time after dissection, the procedure consisted of measuring conductance at some standard concentration (150 or 200 mM) immediately before and after each measurement at other concentrations (0, 18.75, 25, 37.5, 50, 75, 100 and 400 mM) and expressing the conductance as a percentage of the average of the preceding and following conductance values at the standard concentration.

Fig. 3 gives the average conductance values for three gallbladders in $NaNO_3$ solutions (upper part of figure) and for six gallbladders in NaCl solutions (lower part of figure). The conductance-concentration relation is approximately linear, as also found for gallbladder in the cation-selective

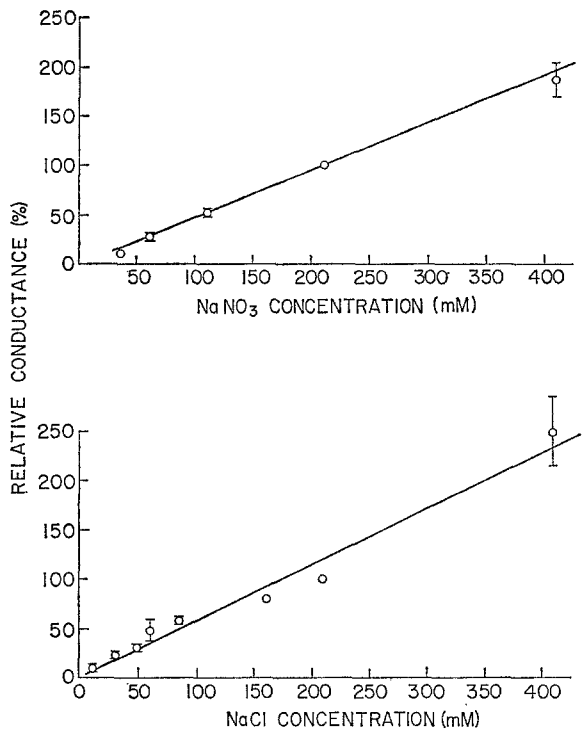


Fig. 3. *Top:* Dependence of gallbladder conductance on bathing salt concentration in symmetrical $NaNO_3$ solutions. [$NaNO_3$] was varied from 25 to 400 mM in the presence of 2.5 mM $Th(NO_3)_4$. All solutions were kept isosmotic with 400 mM $NaNO_3$ + 2.5 mM $Th(NO_3)_4$, by incorporation of mannitol. The ordinate gives the conductance at the given concentration as a percentage of the conductance at 200 mM $NaNO_3$. The abscissa is [$NO_3^-$], which is always 10 mM greater than [$Na^+$] because of the presence of 2.5 mM $Th(NO_3)_4$. Vertical bars give the standard errors of the mean for three experiments. *Bottom:* Same as upper part of figure, except that the results depicted were obtained in symmetrical NaCl solutions, whose concentration was varied from 0 to 400 mM. The abscissa is [NaCl] + 10, to account for the 10 mM of monovalent anion added as 2.5 mM $Th(NO_3)_4$

state at neutral pH (paper III), barnacle muscle (Hagiwara, Toyama & Hayashi, 1971), rabbit intestine (Schultz, Curran & Wright, 1967), frog choroid plexus (Wright, *personal communication*), and nystatin-treated thin lipid membranes (Cass, Finkelstein & Krespi, 1970).

*The Concentration Dependence of Anion-to-Cation Discrimination.* Dilution potentials resulting from 2:1 gradients were measured as a function of absolute concentration in $NaNO_3$, by comparing p.d.'s set up by 25:50, 50:100, 100:200 and 200:400 gradients of $NaNO_3$. The procedure was to hold the serosal solution at 50 mM and measure p.d.'s with 25 or 100 mM $NaNO_3$ in the mucosal solution, then to hold the serosal solution at 100 mM

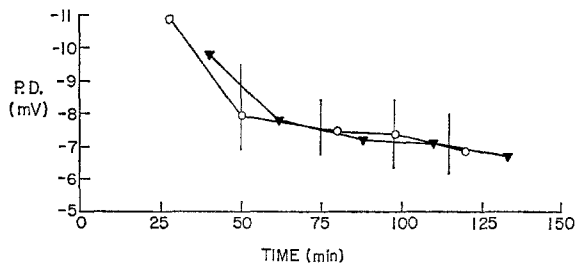


Fig. 4. An experiment designed to measure the cation-to-anion permeability ratio of the gallbladder in the presence of 2.5 mM $Th(NO_3)_4$, as a function of ionic strength. Dilution potentials resulting from 2:1 concentration gradients of $NaNO_3$ were measured at two different absolute concentration levels, 50:25 mM (▾) and 100:50 mM (○), by holding the serosal solution at 50 mM and changing the mucosal solution to 25 mM or to 100 mM. The points ○ have been plotted with reversed sign to facilitate comparison. All solutions were kept isosmotic with 400 mM $NaNO_3$ + 2.5 mM $Th(NO_3)_4$, by incorporation of mannitol. P.d.'s gradually decrease with time but are nearly the same for the two cases; i.e., the dilution potential is approximately independent of absolute concentration and of the direction of the gradient. Interpolated values of pairs of dilution potentials were compared at the times indicated by the vertical lines, and $P_{Na}/P_{NO_3}$ ratios calculated from these p.d.'s are plotted in Fig. 5

and use 50 or 200 mM as the mucosal solution, and finally to hold the serosal solution at 200 mM and use 100 or 400 mM as the mucosal solution. All solutions contained 2.5 mM $Th(NO_3)_4$ and were maintained isosmotic with 400 mM $NaNO_3$ + 2.5 mM $Th(NO_3)_4$ by incorporation of appropriate concentrations of mannitol. As illustrated in Fig. 4, which depicts the p.d.'s measured in one such experiment, the 2:1 dilution potentials are relatively independent of absolute concentration.

Permeability ratios $P_{Na}/P_{NO_3}$ from pairs of dilution potentials measured at the same time were extracted by the Goldman-Hodgkin-Katz equation, and all $P$'s were then normalized to the same time in the experiment by the procedure described in the legend to Fig. 5, to compensate for gradual changes in gallbladder properties with time after dissection. As depicted in Fig. 5, which summarizes $P$'s measured in 12 gallbladders as a function of the average $NaNO_3$ concentration in the two bathing solutions, $P_{Na}/P_{NO_3}$ is 0.27 at 37.5 mM and increases only slightly (by 0.04/100 mM) over the range 37.5 to 300 mM. This permeability ratio is very different from the free-solution mobility ratio, 0.70. Similarly, in cation-selective gallbladders at neutral pH, $P_{Cl}/P_{Na}$ is much lower than the free-solution mobility ratio and increases only slightly with concentration (paper III, Fig. 8).

*The Current-Voltage Relation.* The instantaneous current-voltage relation after $Th^{+4}$ treatment was determined in symmetrical 150 NaCl mM solutions

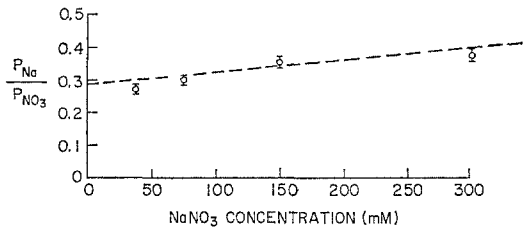


Fig. 5. Anion-to-cation permeability ratios in $Th^{+4}$-treated gallbladder, calculated from the Goldman-Hodgkin-Katz equation, as a function of absolute salt concentration (ionic strength). Dilution potentials resulting from 2:1 gradients of $NaNO_3$ were measured at four different absolute concentration levels (25:50, 50:100, 100:200, and 200:400 mM), as illustrated in the experiment of Fig. 4. To correct for the small variations of p.d.'s with time over the course of an experiment, calculated values of $\alpha \equiv P_{Na}/P_{NO_3}$ were all normalized to the same time within an experiment. This was done by taking the average value of $\alpha$ from the 100:50 mM gradient for the experiment [denoted by $\alpha_{av}(100:50)$], and multiplying each individual $\alpha$ for other gradients by the ratio of $\alpha(100:50)$ interpolated at the same time to $\alpha_{av}(100:50)$. The ordinate gives average $\alpha$ values from 12 different experiments, while the abscissa is the average of the two salt concentrations used to produce the gradient. Vertical bars give the standard errors of the mean. Note that $P_{Na}/P_{NO_3}$ increases only slightly (by 0.04/100 mM) over the concentration range from 37.5 to 300 mM

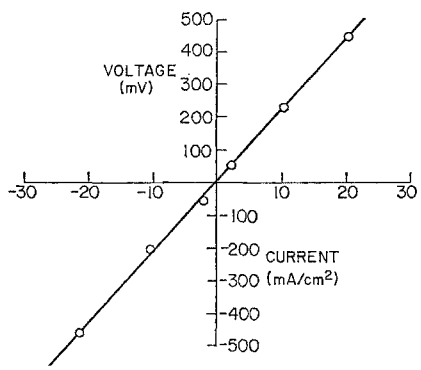


Fig. 6. The instantaneous current-voltage relation of a $Th^{+4}$-treated gallbladder bathed in symmetrical 150 mM NaCl solutions. The voltage drop $\Delta V$ across the gallbladder produced by a constant current was measured within the first 30 msec of onset of the current pulse. To correct for slow variations in conductance with time, $\Delta V$ at a standard current of 2.08 ma/cm$^2$ was measured before and after each measurement at a given density, and experimental $\Delta V$'s were multiplied by the ratio of the interpolated $\Delta V$ for 2.08 ma/cm$^2$ at the same time to the average $\Delta V$ for 2.08 ma/cm$^2$ throughout the experiment. Note that the current-voltage relation is linear

in four gallbladders (current-voltage relations in the same four gallbladders in the cation-selective state have been reported previously: paper III, p. 343). As illustrated in Fig. 6, the relation was linear and symmetrical within $\pm 6\%$ up to the highest voltage tested, 570 mV. In two further gallbladders the

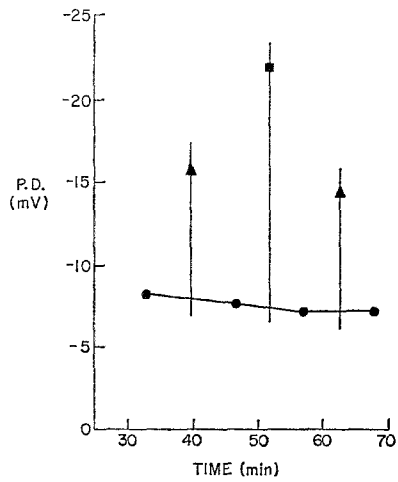


Fig. 7. NaCl dilution potentials as a function of concentration gradient in a $Th^{+4}$-treated gallbladder. The serosal solution was 150 mM NaCl throughout the experiment, and p.d.'s were measured when the mucosal solution was either 75 mM (•), 37.5 mM (▲), or 18.75 mM (■) NaCl to create 2:1, 4:1, and 8:1 gradients. All solutions were maintained isosmotic with 150 mM NaCl + 2.5 $Th(NO_3)_4$ by incorporation of mannitol. Vertical lines indicate times when the p.d. resulting from a 4:1 or 8:1 gradient was compared with the p.d. from a 2:1 gradient

relation was determined in asymmetrical NaCl solutions (150 mM NaCl on one side, 37.5 mM on the other side) and was also linear up to the highest voltage tested, 575 mV. These large voltages appeared to have no damaging effect on the permeability properties of the gallbladder, as indicated by the high values of NaCl dilution potentials still obtained at the end of the experiment. The current-voltage relation is also linear, both in symmetrical and asymmetrical NaCl solutions, in gallbladder in the cation-selective state at neutral pH (paper III) and in frog choroid plexus (Wright, *unpublished observation*).

*Dilution Potentials as a Function of Gradient.* In six $Th^{+4}$-treated gallbladders, dilution potentials resulting from 2:1, 4:1 and 8:1 NaCl concentration gradients were compared. The procedure was to maintain the serosal solution at 150 mM NaCl, and to measure the p.d.'s when the mucosal solution was changed to 75, 37.5 and 18.75 mM NaCl. Fig. 7 illustrates p.d. measurements in one such experiment. In Fig. 8 the average p.d.'s from all six experiments are plotted along with theoretical curves based on four model equations: the Goldman-Hodgkin-Katz equation, thin membrane with fixed neutral sites plus shunt, thick membrane with fixed neutral sites, and thick membrane with fixed neutral sites plus shunt. Each

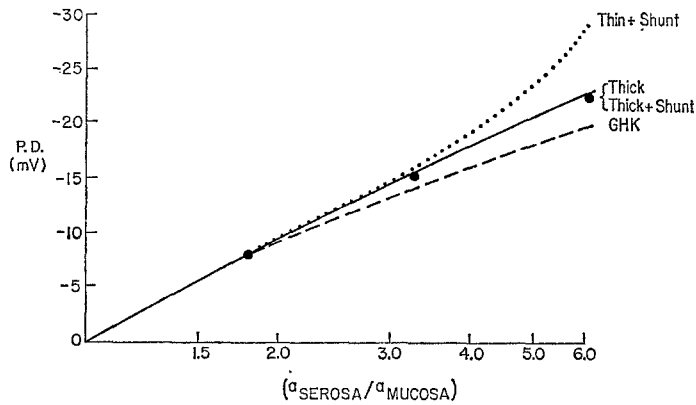


Fig. 8. Comparison of experimental dilution potentials with predictions of four model equations. The experimental points (•) are the average dilution potentials in $Th^{+4}$-treated gallbladders, measured as in Fig. 7. The abscissa is the NaCl activity ratio (activity in serosal solution divided by activity in mucosal solution), plotted on a logarithmic scale. Theoretical curves fitted to pass through the point at an activity ratio of 1.78 (150:75 mM concentration gradient) have been calculated from the model of a thick fixed-neutral-site membrane [———; paper II, Eq. (42)], thick fixed-neutral-site membrane plus shunt [———, curve indistinguishable from previous one; paper II, Eq. (53)], thin fixed-neutral-site membrane plus shunt [·····; paper II, Eq. (110)], and Goldman-Hodgkin-Katz equation [------; paper IV, Eq. (1)]. Note that the fit is good to the thick neutral-site models but is less good to the thin neutral-site model and the Goldman-Hodgkin-Katz equation

theoretical curve is adjusted to go through the experimental point at −7.74 mV, activity ratio 1.78 (2:1 concentration gradient), and the closeness of the curve to the experimental points for 4:1 and 8:1 gradients tests goodness of fit. It is apparent that the experimental points are fitted well by the thick fixed-neutral-site model, with or without shunt, but are fitted less well by the thin fixed-neutral-site model and the Goldman-Hodgkin-Katz equation. An alternative method of testing goodness of fit is to calculate $P_{Na}/P_{Cl}$ from the p.d.'s for 2:1, 4:1 and 8:1 gradients by each equation. The thick fixed-neutral-site model with or without shunt yields values of $P_{Na}/P_{Cl}$ that are nearly independent of gradient, whereas values of $P_{Na}/P_{Cl}$ calculated from the other two models change with gradient.

*Comparison of Dilution Potentials and Biionic Potentials.* Permeability coefficients depend upon some combination of two types of factors: an equilibrium factor, such as a binding constant ($K$) or partition coefficient, and a non-equilibrium factor, such as a mobility ($u$). In a given permeation mechanism the permeability coefficients extracted from dilution potentials, biionic potentials, and conductances may be given by the same or different

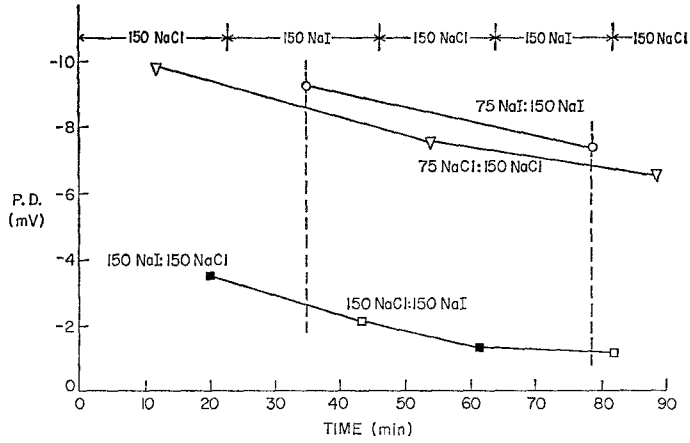


Fig. 9. Comparison of dilution potentials and biionic potentials in NaCl and NaI. P.d.'s were measured when a $Th^{+4}$-treated gallbladder separated the following pairs of solutions, listing the mucosal solution first and the serosal solution second in each case: dilution potentials, 75 mM NaCl *vs.* 150 mM NaCl (▽), 75 mM NaI *vs.* 150 mM NaI (○); biionic potentials, 150 mM NaI *vs.* 150 mM NaCl (▪), 150 mM NaCl *vs.* 150 mM NaI (□). The ordinate gives the potential of the mucosal solution with respect to that of the serosal solution, except that the potential of points ▪ has been reversed in sign to facilitate comparisons. The composition of the serosal solution is indicated by the arrows at the top of the figure. Vertical broken lines indicate times at which dilution potentials and biionic potentials were compared

combinations of $u$'s and $K$'s. For instance, in thin membranes with neutral sites, whether fixed (paper II) or mobile (Ciani, Eisenman & Szabo, 1969), relative permeability coefficients for the $i^{th}$ ion are given by the combination $u_i K_i^{1/n}$ whether calculated from dilution potentials, biionic potentials, or conductances, while in a thick ion-exchange membrane dilution potentials and conductances depend solely on $u$'s but biionic potentials depend on both $u$'s and $K$'s. Comparison of $P$'s extracted from dilution potentials and biionic potentials therefore provides a clue to the permeation mechanism.

A previous section (pp. 73–76) discussed experiments comparing biionic potentials for eight different anions, interpolated to 25 min after dissection. The experiments discussed in this section involve comparison of dilution potentials and biionic potentials for a given pair of salts, interpolated to the same time in an experiment but not necessarily at 25 min after dissection. It was feasible to compare only two, or rarely, three salts in a given experiment. Fig. 9 illustrates the protocol and interpolation procedure in one experiment in which NaCl and NaI were compared. Table 5 presents the p.d.'s measured in each individual experiment, along with $P$'s extracted from the equations describing a fixed-neutral-site mem-

Table 5. P.d.'s and anion permeability ratios in individual experiments

| Anions | Exp. no. | P.d.'s (mV) | | | Permeability ratios | |
|---|---|---|---|---|---|---|
| | | Dilution potentials | | Biionic potentials | From dilution potentials | From biionic potentials |
| | | $E_{NaCl}$ | $E_{NaSCN}$ | $E_{NaCl:NaSCN}$ | $P_{SCN}/P_{Cl}$ | $P_{SCN}/P_{Cl}$ |
| Cl:SCN | 1 | −8.95 | −9.20 | 4.57 | 1.11 | 1.46 |
| | 2 | −6.90 | −8.10 | 7.45 | 1.68 | 2.22 |
| | 3*a* | −8.78 | −10.25 | 4.76 | 1.69 | 1.49 |
| | 3*b* | −6.69 | −7.40 | 0.27 | 1.45 | 1.22 |
| | | | | | 1.48 ± 0.14 avg | 1.59 ± 0.22 |
| | | $E_{NaCl}$ | $E_{NaNO_3}$ | $E_{NaCl:NaNO_3}$ | $P_{NO_3}/P_{Cl}$ | $P_{NO_3}/P_{Cl}$ |
| $Cl:NO_3$ | 4*a* | −10.36 | −9.95 | 2.97 | 0.96 | 1.27 |
| | 4*b* | −8.72 | −7.40 | 0.10 | 0.82 | 1.12 |
| | 5*a* | −9.72 | −10.55 | 2.77 | 1.57 | 1.28 |
| | 5*b* | −8.20 | −8.00 | 0.40 | 1.05 | 1.14 |
| | 5*c* | −9.70 | −11.55 | 6.20 | 2.52 | 1.57 |
| | 6 | −8.90 | −8.75 | 0.60 | 1.06 | 1.14 |
| | | | | | 1.33 ± 0.26 avg | 1.25 ± 0.07 |
| | | $E_{NaCl}$ | $E_{NaBr}$ | $E_{NaCl:NaBr}$ | $P_{Br}/P_{Cl}$ | $P_{Br}/P_{Cl}$ |
| Cl:Br | 4*c* | −8.60 | −8.90 | 0.97 | 1.08 | 1.08 |
| | 7 | −8.60 | −10.00 | 2.95 | 1.62 | 1.25 |
| | 8*a* | −9.40 | −11.90 | 1.55 | 2.71 | 1.11 |
| | 8*b* | −9.35 | −9.10 | 1.15 | 0.88 | 1.08 |
| | | | | | 1.57 ± 0.41 avg | 1.13 ± 0.04 |
| | | $E_{NaCl}$ | $E_{NaClO_4}$ | $E_{NaCl:NaClO_4}$ | $P_{ClO_4}/P_{Cl}$ | $P_{ClO_4}/P_{Cl}$ |
| $Cl:ClO_4$ | 9*a* | −9.40 | −9.60 | 6.20 | 1.21 | 1.58 |
| | 9*b* | −7.10 | −6.40 | −1.05 | 0.92 | 1.04 |
| | 10*a* | −9.40 | −9.50 | 5.15 | 1.16 | 1.49 |
| | 10*b* | −7.55 | −7.55 | −0.70 | 1.17 | 1.07 |
| | | | | | 1.12 ± 0.07 avg | 1.24 ± 0.14 |
| | | $E_{NaCl}$ | $E_{NaI}$ | $E_{NaCl:NaI}$ | $P_I/P_{Cl}$ | $P_I/P_{Cl}$ |
| Cl:I | 11*a* | −9.20 | −10.00 | 4.95 | 1.29 | 1.38 |
| | 11*b* | −7.17 | −7.85 | 1.00 | 1.25 | 1.09 |
| | 12*a* | −8.60 | −9.25 | 2.65 | 1.22 | 1.21 |
| | 12*b* | −6.85 | −7.40 | 1.20 | 1.20 | 1.12 |
| | | | | | 1.24 ± 0.02 avg | 1.20 ± 0.07 |
| | | $E_{NaBr}$ | $E_{NaNO_3}$ | $E_{NaBr:NaNO_3}$ | $P_{Na_3}/P_{Br}$ | $P_{NO_3}/P_{Br}$ |
| $Br:NO_3$ | 13*a* | −9.65 | −10.10 | 0.45 | 1.38 | 1.12 |
| | 13*b* | −7.95 | −7.05 | −1.25 | 1.12 | 1.00 |
| | 14*a* | −11.15 | −11.00 | 0.00 | 1.13 | 1.07 |
| | 14*b* | −9.25 | −8.85 | −0.25 | 1.00 | 1.07 |
| | | | | | 1.15 ± 0.08 avg | 1.06 ± 0.02 |

brane with shunt. These values of the $P$'s are considered the most physically realistic ones (pp. 90–91). The average values of $P$'s extracted from four different model equations are summarized in Table 6.

Three preliminary conclusions may be drawn from Tables 5 and 6. First, the anion permeability sequences implied by the biionic potentials of Tables 5 and 6 are not identical to the sequence derived in Table 3 from biionic potentials at 25 min after dissection, because the sequence changes with time after dissection (p. 76, Fig. 2 and Table 4), and the p.d. measurements of Table 5 were generally obtained at times other than 25 min. Second, $P$'s extracted from biionic potentials agree well with $P$'s extracted from dilution potentials, except in the case of the $Br^- - Cl^-$ pair. Finally, comparison of $P$'s from different equations in Table 6 shows (a) that the Goldman-Hodgkin-Katz equation and thick fixed-neutral-site model without shunt yield nearly the same $P$'s, despite the very different form of these two equations; (b) the thin fixed-neutral-site model with shunt and the thick-neutral-site model with shunt yield almost identical $P$'s; and (c) the thick model plus shunt (and the thin model plus shunt) yields higher $P$'s than does the thick model without shunt (or the Goldman-Hodgkin-Katz equation), because correction for the shunt shifts $P$'s in the direction away from the free-solution mobility ratios. The effect is most marked for $P_{SCN}/P_{Cl}$, since this is the ion pair whose permeability ratio is furthest removed from the free-solution mobility ratio. Similar conclusions concerning the significance of $P$'s extracted from different model equations were reached in the analysis of cation permeation in the gallbladder (paper IV, pp. 385–389).

To Table 5:

This table lists the results of all individual experiments in which permeabilities of two anions were compared by measuring dilution potentials and biionic potentials. Column 1 gives the two anions compared; column 2, the number of the experiment; columns 3 and 4, the dilution potential for a 150:75 mM gradient of each $Na^+$ salt; column 5, the biionic potential between 150 mM solutions of the two $Na^+$ salts, listing the one in the serosal solution first and the one in the mucosal solution second (since the p.d. is given as the potential of the mucosal solution with respect to that of the serosal solution, a negative sign means that the first anion is more permeant than the second, a positive sign, vice versa); column 6, the permeability ratio extracted from the two dilution potentials by the equation for a thick fixed-neutral-site membrane with shunt ($u_1(K_1K_3)^{1/2n}/\lambda_s A_n v_3$ was calculated for each salt from paper II, Eq. (53), setting $u_3=0$ and $n=1$, and the ratio was taken of the values for the two salts); and column 7, the permeability ratio extracted from the biionic potential by the equation for a thin fixed-neutral-site membrane with shunt ($u_2K_2^{1/n}/u_1K_1^{1/n}$ in paper II, Eq. (113), setting $u_3=0$ and $n=1$). Average permeability ratios ("avg") are also listed for each ion pair.

Table 6. Comparison of anion permeability ratios calculated from four model equations

| | GHK | | Thin + Shunt | | Thick | | Thick + Shunt |
|---|---|---|---|---|---|---|---|
| | Dilution | Biionic | Dilution | Biionic | Dilution | Biionic | Dilution |
| $P_{SCN}/P_{Cl}$ | 1.19 ± 0.07 | 1.24 ± 0.09 | 1.49 ± 0.14 | 1.59 ± 0.22 | 1.18 ± 0.08 | 1.24 ± 0.09 | 1.48 ± 0.14 |
| $P_{NO_3}/P_{Cl}$ | 1.22 ± 0.17 | 1.13 ± 0.15 | 1.33 ± 0.32 | 1.25 ± 0.07 | 1.21 ± 0.18 | 1.14 ± 0.12 | 1.33 ± 0.26 |
| $P_{Br}/P_{Cl}$ | 1.38 ± 0.25 | 1.11 ± 0.04 | 1.57 ± 0.41 | 1.13 ± 0.04 | 1.39 ± 0.28 | 1.06 ± 0.03 | 1.57 ± 0.41 |
| $P_{ClO_4}/P_{Cl}$ | 1.01 ± 0.04 | 1.13 ± 0.11 | 1.12 ± 0.07 | 1.24 ± 0.14 | 1.02 ± 0.04 | 1.15 ± 0.11 | 1.12 ± 0.07 |
| $P_I/P_{Cl}$ | 1.10 ± 0.03 | 1.12 ± 0.04 | 1.23 ± 0.02 | 1.20 ± 0.07 | 1.11 ± 0.03 | 1.12 ± 0.05 | 1.24 ± 0.02 |
| $P_{NO_3}/P_{Br}$ | 1.07 ± 0.04 | 1.04 ± 0.02 | 1.15 ± 0.08 | 1.06 ± 0.02 | 1.09 ± 0.06 | 1.06 ± 0.07 | 1.15 ± 0.08 |

Anion permeability ratios $P_{X^-}/P_{Y^-}$ were calculated from pairs of dilution potentials (as the ratio of $P_{X^-}/P_{Na^+}$ to $P_{Y^-}/P_{Na^+}$) and from biionic potentials, for all the individual experiments iisted in Table 5, and the results were averaged. The equations used were: the Goldman-Hodgkin-Katz equation [paper IV, Eq. (1)]; the equation for a thin fixed-neutral-site membrane plus shunt [paper II, Eq. (113), $u_2 K_2^{1/n}/u_1 K_1^{1/n}$, for biionic potentials, and paper II, Eq. (110), $P_{anion}/P_{Na^+}$ calculated as $u_1 K_1^{1/n}/\lambda_s v_3$, for dilution potentials, taking $u_3 = 0$ and $n = 1$]; the equation for a thick fixed-neutral-site membrane without shunt [paper II, Eq. (43), $u_2 K^{1/n}/u_1$, for biionic potentials, and paper II, Eq. (42), $P_{anion}/P_{Na^+}$ calculated as $u_1/u_3$, for dilution potentials, taking $n = 1$]; and the equation for a thick fixed-neutral-site membrane plus shunt [paper II, Eq. (53), $P_{anion}/P_{Na^+}$ calculated as $u_1 K_1 K_3^{1/2u}/\lambda_s A_n v_3$, taking $u_3 = 0$ and $n = 1$, for dilution potentials; biionic potential equation too complex to use]. *See* text, p. 85, for discussion.

## Discussion

The Discussion section considers the three subjects of the Results section in a different order: anion selectivity; the mechanism of anion permeation in $Th^{+4}$-treated gallbladders; and the action of $Th^{+4}$.

### *Anion Selectivity*

The anion permeability sequence at 20 min after exposure to $Th^{+4}$, the earliest time at which comparisons could be made, is $SCN^- > ClO_4^- > NO_3^- > NO^- > I^- > Br^- \geqq ClO_3^- > Cl^- > BrO_3^-$. This sequence differs considerably from the mobility sequence in free solution, $Br^-$ (78.1) > $I^-$ (76.8) > $Cl^-$ (76.4) > $NO_3^-$ (71.4) > $ClO_4^-$ (67.4) > $SCN^-$ (66) > $ClO_3^-$ (64.6) > $BrO_3^-$ (55.8) (the numbers in parentheses are limiting equivalent conductivities at infinite dilution). It also differs from the sequence of enthalpies or free energies of hydration, the sequence of ionic radii, the sequence of so-called "hydrated radii", and the so-called lyotropic series.

Similar sequences of specific ion effects, differing from both the ionic-radius sequence and the hydration energy sequence, have been observed in numerous biological as well as non-living systems (Eisenman, 1961, 1962; Diamond & Wright, 1969). Examples of natural processes with anion specificities similar to the permeability sequence in $Th^{+4}$-treated gallbladder include permeation in barnacle muscle at low pH (Hagiwara *et al.*, 1971: $SCN^- > I^- > NO_3^- > Br^- > ClO_3^- > Cl^- > BrO_3^-$, as the gallbladder except for the $NO_3^- - I^-$ inversion), effects on potentials at an air/water interface (Haydon, 1964: $SCN^- > ClO_4^- > I^- > NO_3^- > Br^- > Cl^-$), and effects on the isoionic point of serum albumin (Scatchard & Black, 1949: $SCN^- \sim ClO_4^- > NO_3^- \geqq I^- > Br^- > Cl^-$). Eisenman (1961, 1962) showed that biological selectivity patterns for the alkali cations could be predicted by comparing the cations' hydration energies with their Coulomb interaction energies with membrane negative sites, and that the controlling variable was the effective field strength of the sites. Diamond and Wright (1969), confirming predictions by Eisenman (1965), found that a similar approach sufficed to interpret all known biological selectivity patterns for the halide anions and that there were quantitative regularities in halide selectivity that permitted the construction of so-called empirical selectivity isotherms. The relative permeability coefficients for $I^-$, $Br^-$, and $Cl^-$ listed in Table 3 fit very well on the selectivity isotherms constructed from measurements in other biological systems (Diamond & Wright, 1969, Fig. 1). The gallbladder sequence $I^- > Br^- > Cl^-$ is one of the common biological sequences termed sequence 1 in the terminology of Diamond and Wright. This sequence implies "weak sites", i.e., membrane positive sites whose field strength is small compared to the field of a water molecule, such that differences in the hydration energies of different halides exceed differences in the interaction energies of the halides with the site. Biological systems also exhibit quantitative regularities in discriminating polyatomic monovalent anions such as $ClO_4^-$, $SCN^-$, $NO_3^-$, $ClO_3^-$, and $BrO_3^-$, and the gallbladder ratios also fit well with selectivity isotherms constructed for these ions (Diamond, 1972).

### *The Mechanism of Anion Permeation in $Th^{+4}$-Treated Gallbladder*

*The Rate-Controlling Membrane is Thick Enough that Microscopic Electroneutrality Has to be Obeyed.* This conclusion follows from the finding that the current-voltage relation is linear in both symmetrical and asymmetrical NaCl solutions up to at least 570 mV. As discussed previously in connection with the same finding in cation-selective gallbladders (papers II, III and IV), membranes which are thin compared to their Debye length exhibit

a non-linear current-voltage relation in asymmetrical single-salt solutions if ions permeate via fixed neutral sites such as a polar pore, and a non-linear relation is exhibited in symmetrical solutions as well if ions are solubilized in the low-dielectric membrane interior, e.g., by a neutral carrier.

*The Permeation Mechanism is Not the Same as in a Classical Ion-Exchanger.* At salt concentrations up to at least 400 mM, $Th^{+4}$-treated gallbladders exhibit a linear conductance-concentration relation, a finite permeability to both cations and anions, and an anion-to-cation permeability ratio that is relatively independent of concentration and differs markedly from the free-solution mobility ratio. This combination of properties is very unlike those of a macroscopic, hydratable, single-channel ion-exchanger (Teorell, 1935, 1951, 1953; Meyer & Sievers, 1936), in which cation-anion discrimination depends principally upon an imbalance between the concentrations of mobile cations and mobile anions in the membrane, as a result of the presence of fixed-charge sites and the electroneutrality condition. In solutions with salt concentrations much lower than the fixed-charge density, the conductance of such an ion-exchanger is nearly independent of concentration, and co-ions are virtually impermeant. A linear conductance-concentration relation is obtained only when bathing-solution salt concentrations considerably exceed site concentrations, but the permeability ratios generally approach free-solution mobility ratios under these conditions. From ion-exchange theory (e.g., Teorell, 1953), one may calculate that the site concentration necessary to account for the 150:75 mM NaCl dilution potential in the gallbladder would be 191 mM (positive sites) after $Th^{+4}$ treatment and 530 mM (negative sites) at neutral pH, assuming mobility ratios as in free solution. Over the experimental concentration range of 25 to 400 mM used in the gallbladder experiments, these site densities would result in markedly non-linear conductance-concentration relations and concentration-dependent cation-anion permeability ratios. Mosaic ion-exchangers do exhibit in dilute solutions a relatively concentration-independent cation-to-anion permeability ratio differing from the free-solution mobility ratio, but are still characterized by a relatively concentration-independent conductance in this concentration range.

It is perhaps possible, however, that an ion-exchanger could discriminate between cations and anions principally as a result of ion mobility differences rather than ion concentration differences within the membrane. Specifically, if the membrane structure was such that the presence of a few charged sites somehow caused counter-ion mobility greatly to exceed co-ion mobility even in solutions with salt concentrations much greater than the site density,

then one would obtain both an approximately linear conductance-concentration relation and an approximately concentration-independent cation-to-anion permeability ratio differing from the free-solution mobility ratio. Such a system would behave formally like a neutral-site membrane. We do not know of an actual cation-exchanger in which $u_K$ exceeds $u_{Cl}$ by at least a factor of 30, as in gallbladder at neutral pH, but the possibility cannot be rejected and is mentioned again on p. 94.

*The Permeation Mechanism is Probably Located in the Tight Junctions.* Several of the lines of evidence (Frömter & Diamond, 1972; paper IV; Diamond, Barry & Wright, 1971; Frömter, 1972; Machen *et al.*, 1972) which indicated that the high-conductance pathway for cation permeation in the gallbladder at neutral pH resides in the tight junctions between cells, rather than in the epithelial cell membranes, also apply to anion permeation in $Th^{+4}$-treated gallbladders. Strong evidence is provided by the linear current-voltage relation, which signifies a rate-controlling membrane that obeys microscopic electroneutrality and is thick compared to a 80-Å lipid bilayer or cell membrane. Since the membrane-fusion region of gallbladder tight junctions is ca. 2000 to 3000 Å thick (Tormey & Diamond, 1967) but the epithelial cell membranes are typical 80-Å cell membranes, the current-voltage relation is the one expected for the tight junctions and would be difficult to reconcile with the epithelial cell membranes. Other features suggestive of or consistent with a tight-junction route are the presence of $Th^{+4}$ in the tight junctions (Tormey, *unpublished observation*); the low transepithelial resistance; and the symmetry of diffusion potentials, such that reversing mucosal and serosal bathing solutions yields a p.d. of opposite sign but the same magnitude.

*The Sites Controlling Anion Permeation Are Probably Fixed, Not Mobile.* The sites might either be located on carriers within a hydrocarbon-like membrane, or else fixed within a polar pore. In the former case it would be difficult to explain the presence and low mobility of the cations needed to maintain microscopic electroneutrality in a thick membrane (*cf.* paper IV, pp. 377–378). The latter interpretation agrees with the evidence for a tight-junction permeation route, which would provide a polar pore rather than a hydrocarbon milieu.

*Cation Permeation is Mainly Via a Free-Solution Shunt.* In freshly dissected gallbladders exposed to $Th^{+4}$ for 7 to 15 min, sodium conductance ($G_{Na}$) is low, as indicated by the value of $P_{Na}/P_{Cl}=0.13$ calculated from dilution potentials. Most of this small sodium conductance is in the free-solution shunt that exists even prior to exposure to $Th^{+4}$ (p. 71–73). The

gradual changes in dilution potentials and conductance, including a four-fold increase in $G_{Na}$, that occur during 2 hr of exposure to $Th^{+4}$ may be entirely accounted for by an increase in the shunt (Fig. 1). Thus, most of the $G_{Na}$ in $Th^{+4}$-treated gallbladders is in the shunt. This interpretation is supported by two other findings. First, cation permeabilities in anion-selective gallbladders at low pH are in or near the sequence of the free-solution mobilities (Wright & Diamond, 1968). Second, dilution potentials and biionic potentials yield essentially the same anion permeability ratios (Table 6), whereas this should in general not be the case in a thick fixed-neutral-site membrane without shunt, since dilution potentials would depend solely on $u$'s but biionic potentials would depend on the product of $K$'s and $u$'s (paper II). Evidently cation permeability is negligible in the native-membrane channel which is modified by $Th^{+4}$ and through which anions permeate.

*A Mathematical Description of Permeation.* The experimental findings thus suggest that ion permeation in $Th^{+4}$-treated gallbladder is by way of a membrane thick enough to have to obey microscopic electroneutrality, that this membrane is to be identified with the tight junctions, that the sites controlling permeation are fixed, that cation permeability in the native membrane is negligible, and that a free-solution shunt which increases with time is in parallel with this channel. All the permeability properties of $Th^{+4}$-treated gallbladders described on pp. 76–86 are qualitatively identical to those of untreated, cation-selective gallbladders, except for the inversion of permselectivity. These properties (notably the linear conductance-concentration relation, and the anion-to-cation permeability ratio relatively independent of concentration) agree with those expected for a membrane with neutral sites. As discussed on pp. 92–93, however, there are configurations in which a membrane with charged sites could exhibit behavior mimicking a thick fixed-neutral-site membrane.

A mathematical treatment of a thick fixed-neutral-site membrane with shunt was derived in paper II. Eq. (53) of paper II has been used to extract permeability ratios from dilution potentials in the individual experiments tabulated in Table 5. As seen in Fig. 8, this model equation provides a good fit to the experimental relation between dilution potentials and concentration gradient. The equation describing biionic potentials in a thick fixed-neutral-site membrane with shunt [paper II, Eq. (62)] is too complex for use in calculating $P$'s. We have therefore extracted $P$'s from biionic potentials by Eq. (113) of paper II, the corresponding equation for a thin membrane with fixed neutral sites and shunt. $P$'s extracted from dilution potentials by the

thick-membrane model and the thin-membrane model are practically identical (Table 6), and good agreement is also expected in the case of biionic potentials (*see* paper IV, p. 383, for further discussion).

### *The Mechanism of the $Th^{+4}$ Effect*

The effect of $Th^{+4}$ in converting the gallbladder from cation-selective to anion-selective involves both a decrease in cation conductance and an increase in anion conductance, as is true of the effect of $H^+$ on the gallbladder (Wright & Diamond, 1968) and on barnacle muscle (Hagiware *et al.*, 1971). Similarly, $H^+$ and $Th^{+4}$ convert ion-exchangers from cation-selective to anion-selective by decreasing cation conductance and increasing anion conductance (Helfferich, 1962). The effect in ion-exchangers involves a change in the relative concentration of mobile cations and mobile anions in the membrane: $H^+$ and $Th^{+4}$ neutralize negatively-charged groups serving as fixed counterions for cations, and are bound and thereby create positively-charged groups serving as fixed counter-ions for anions. However, the gallbladder, both under physiological, cation-selective conditions and in the $Th^{+4}$-treated[1], anion-selective state, behaves as a neutral-site membrane, not as classical ion-exchanger (p. 88). How is this surprising result to be explained?

Three alternative types of mechanisms will be suggested whereby $Th^{+4}$ could convert the gallbladder from cation-selective to anion-selective while yielding properties formally similar to those of a neutral-site membrane. Other hypotheses[2] are also possible, but the following three seem to us the most likely ones:

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1 It should be remembered that the "$Th^{+4}$ effect" is due only in part to $Th^{+4}$ itself, in part to other ionic species. First, addition of 2.5 mM $Th^{+4}$ to the experimental salt solutions lowers the pH to 3.0. The effect of this $H^+$ concentration (1 mM) in the absence of $Th^{+4}$ on gallbladder conductance is qualitatively the same as, though quantitatively less marked than, the effect of 2.5 mM $Th^{+4}$ solutions. Part, but not all, of the effect of $Th^{+4}$ is therefore actually an effect of $H^+$, and quantitative comparison suggests that $Th^{+4}$ and $H^+$ are approximately equally potent on a molar basis. Second, in aqueous solution some $Th^{+4}$ exists in the form of its hydrolysis products, such as $Th(OH)_3^+$ and $Th(OH)_2^{+2}$ (Sillen & Martell, 1964), which may be responsible for some of the observed effects.

2 We have also considered mechanisms which involve $Th^{+4}$ binding to or screening surface charges, thereby increasing the anion concentration and decreasing the cation concentration at the membrane: solution interface (McLaughlin *et al.*, 1970; McLaughlin, Szabo & Eisenman, 1971). However, the linear conductance-concentration relation in both the cation-selective state and the anion-selective state is not readily explained by such mechanisms without further assumptions.

(1) *Many Thin Ion-Exchange Elements in Series* (Fig. 10*a*). High-resolution electron micrographs of the tight junction suggest that it may consist of a mesh of channels which are much narrower in some places than in others (Brightman & Reese, 1969; Goodenough & Revel, 1970). Most of the electrical resistance could reside in these narrow regions, each of which represents only a small fraction of the total length of the junction. If these regions were lined by negative groups ($-COO^-$) at neutral pH and by positive groups ($-NH^+$ or $Th^{+4}$) at low pH or in the presence of $Th^{+4}$, such a system would behave as a series of discrete, thin membranes with fixed ion-exchange groups.

The black-box properties of the system illustrated in Fig. 10*a* might resemble more closely those of a thick fixed-neutral-site membrane than either those of a classical ion-exchanger or those of a single thin ion-exchanger. If each resistive element is thin compared to the Debye length so that microscopic electroneutrality is not obeyed, the concentration of counterions could be approximately propertional to the salt concentration in the adjacent solutions, as in a neutral-site membrane, rather than equal to the fixed-charge concentration, as in a classical ion-exchanger. A linear conductance-concentration relation and a concentration-independent anion-to-cation permeability ratio would result (given co-ion conductance in a parallel shunt). The current-voltage relation would be non-linear if a voltage of 570 mV and a 4:1 salt concentration gradient were present across a single thin resistive element. However, the relation approaches linearity for decreasing voltages and decreasing gradients (paper II, Fig. 7). If the whole junction consisted of many separated narrow regions, the fraction of the voltage and of the gradient across each element would be small, and hence the deviations from linearity would be slight.

(2) *Ion-Exchanger in Series with Fixed-Neutral-Site Membrane* (Fig. 10*b*). Imagine an ion-exchanger with a negative charge (e.g., due to $-COO^-$) at neutral pH, hence offering a much higher resistance to anions $R'_-$ than to cations $R'_+$. In series with the ion-exchanger is a fixed-neutral-site membrane, sufficiently long that its resistance to cations $R''_+$ is much greater than $R'_+$ but is still much less than $R'_-$. Then the overall system would be preferentially permeable to cations (since $R'_- \gg R'_+ + R''_+$), but black-box characteristics of cation permeation (such as the conductance-concentration relation or the current-voltage relation) would be dominated by the neutral-site region (since $R''_+ \gg R'_+$). The system would behave formally like a fixed-neutral-site membrane with low anion mobility. If the ion-exchange "gate" acquired a positive charge (due to $Th^{+4}$ or $-NH_3^+$) such that $R'_+ \gg R''_- \gg R'_-$, the system would behave like a neutral-site membrane with low cation mobility.

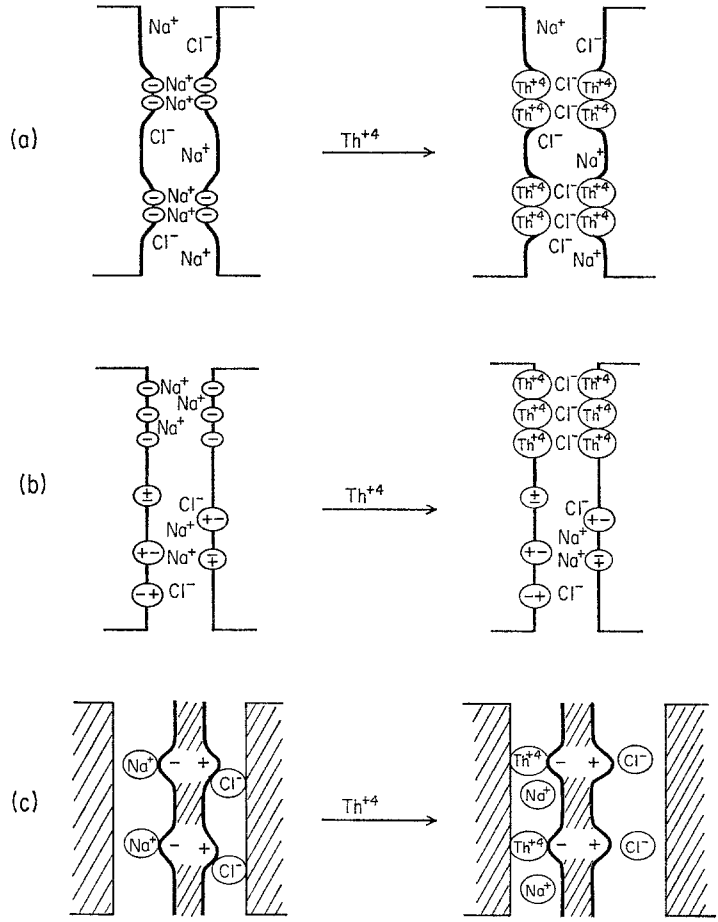


Fig. 10*a–c*. Three models suggesting how a membrane could be cation-selective in the absence of $Th^{+4}$, anion-selective in the presence of $Th^{+4}$, and still behave as a fixed-neutral-site membrane under both conditions. (*a*) Thin ion-exchange elements in series: a pore has several narrow regions which account for most of its electrical resistance, which are lined with negative charges and act as cation-exchangers (*left*), and each of which is short compared to the Debye length. $Th^{+4}$ converts the narrow regions to anion-exchangers (*right*). (*b*) Ion-exchanger and fixed-neutral-site membrane in series: a pore contains an anion-impermeable region of negative fixed charges and a region of dipolar groups in series, so that the pore is cation-selective but most of the resistance to cations is in the dipolar region (*left*). $Th^{+4}$ converts the former region to a cation-impermeable zone of positive fixed charges (*right*). (*c*) A pore with dipolar groups has low anion mobility because anions are more tightly bound and/or have a narrower pathway than cations (*left*). $Th^{+4}$ inverts the anion-to-cation mobility ratio, e.g., by blocking the cation pathway and distorting the pore to open further the anion pathway (*right*). *See* text, pp. 92–94, for further details

(3) *Mobility Differences.* The preceding two models invoke fixed sites with net charge, arranged in such a way that the black-box properties of the whole system resemble those of a neutral-site membrane. Alternatively, at neutral pH the permeation channel may actually contain dipolar groups without net charge (such as $>C=O$ or else an amino acid bearing both $-COO^-$ and $-NH_3^+$) and thus actually constitute a fixed neutral-site membrane, in which anion-cation discrimination depends upon mobility differences rather than concentration differences (*cf.* p. 88). Anion mobility lower than cation mobility could result either from stronger binding between anions and the positive end of the dipole than between cations and the negative end, or else from steric factors (a more constricted pathway for anions). The effect of $Th^{+4}$ binding to the negative end of the dipole would be to invert the cation-to-anion mobility ratio: for example, by blocking the cation pathway and opening the anion pathway further.

It is not possible at present to decide which of these three models is closest to the actual detailed mechanism of $Th^{+4}$ or $H^+$ action. At least three other epithelia (small intestine, choroid plexus, renal proximal tubule) closely resemble the gallbladder in that transepithelial ion permeation is mainly via the tight junctions, cation selectivity at neutral pH apparently involves the fixed-neutral-site mechanism, and low pH inverts the cation-to-anion permeability ratio. The $Th^{+4}$- and $H^+$-induced selectivity inversion analyzed in this paper for the gallbladder may thus be a common phenomenon for epithelia with "leaky" tight junctions.

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