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The Mechanism of Cation Permeation in Rabbit Gallbladder

Conductances, the Current-Voltage Relation, the Concentration Dependence of Anion-Cation Discrimination, and the Calcium Competition Effect

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Summary. The questions underlying ion permeation mechanisms, the types of experiments available to answer these questions, and the properties of some likely permeation models are examined, as background to experiments designed to characterize the mechanism of alkali cation permeation across rabbit gallbladder epithelium. Conductance is found to increase linearly with bathing-solution salt concentrations up to at least 400 mM. In symmetrical solutions of single alkali chloride salts, the conductance sequence is $K^+ > Rb^+ > Na^+ > Cs^+ \sim Li^+$. The current-voltage relation is linear in symmetrical solutions and in the presence of a single-salt concentration gradient up to at least 800 mV. The anion/cation permeability ratio shows little change with concentration up to at least 300 mM. Ca^{++} reduces alkali chloride single-salt dilution potentials, the magnitude of the effect being interpreted as an inverse measure of cation equilibrium constants. The equilibrium-constant sequence deduced on this basis is $K^+ > Rb^+ > Na^+ \sim Cs^+ \sim Li^+$. These results suggest (1) that the mechanism of cation permeation in the gallbladder is not the same as that in a macroscopic ion-exchange membrane; (2) that cation mobility ratios are closer to one than are equilibrium-constant ratios; (3) that the rate-limiting step for cation permeation is in the membrane interior rather than at the membrane-solution interface; and (4) that the rate-controlling membrane is one which is sufficiently thick that it obeys microscopic electroneutrality.

This and the following paper (Barry, Diamond & Wright, 1971 – referred to hereafter as paper IV), the third and fourth in a series of four, report experiments designed to characterize the mechanism of alkali cation permeation across gallbladder epithelium. Earlier experiments (Wright & Diamond, 1968) established that the ion permeability sequence in the gallbladder differs markedly from the free-solution mobility sequence, and fits qualitatively into the regular pattern of alkali cation selectivity sequences which encompasses most living and nonliving systems (Eisenman, 1962, 1965; Diamond & Wright, 1969). Thus, the $Cl^-:K^+$ permeability ratio is

about 0.1, as compared to a free-solution mobility ratio of 1. Among the alkali cations, the permeabilities to Cs^+ and Rb^+ fall out of the free-solution sequence with respect to the other cations, and the permeability ratios for K^+, Na^+, and Li^+ differ quantitatively from the free-solution mobility ratios, although they stand in qualitatively the same sequence. The effect of pH in changing cation permeability ratios and sequences in the gallbladder is similar to the effect in glass electrodes (Wright & Diamond, 1968, p. 72; Diamond & Wright, 1969, p. 592) and is in accord with Eisenman's successful reconstruction of cation selectivity patterns from simple physical principles.

Gallbladder epithelium consists of a single layer of cells held together by so-called tight junctions, which are membrane-fusion areas completely encircling each cell (Barry & Diamond, 1970, Fig. 1). The first paper in this series (Barry & Diamond, 1970—referred to hereafter as paper I) demonstrated that transepithelial permeation is controlled by a single membrane, implying either that the cell membrane at one face of the epithelial cells has a negligible resistance compared to that at the other face, or else that the high-conductance pathway across the epithelium is via the tight junctions, bypassing the cell interior. As will be discussed, the experimental results favor the latter interpretation.

In the absence of knowledge of the complete molecular structure of a given membrane, one may still attempt to deduce its ion permeation mechanisms by comparing its "black-box" characteristics of ion permeation with the "black-box" properties of model membranes in which structural details and permeation mechanisms are well understood. In order to provide a framework for understanding the experiments and discussion, this introductory section therefore examines, in turn, some questions involved in ion permeation mechanisms, the types of "black-box" experiments performed to answer these questions, and the principal relevant models of ion permeation. The experimental results included in the present paper consist of: (a) the current-voltage relation; (b) the effect of concentration on conductance, and on the anion/cation permeability ratio (this pair of experiments provides a crucial test of a macroscopic ion-exchanger permeation mechanism); and (c) the cation selectivity sequence as deduced from conductances, and as deduced from the Ca^{++} competition effect. The following paper presents the electrical potential experiments [dilution potentials and biionic potentials as a function of concentration gradient, and the cation selectivity sequences deduced from these two kinds of potential differences (p. d.)] and their detailed analysis, and formulates a tentative model of the permeation mechanism.

Questions Underlying Ion Permeation Mechanisms

Identifying the ion permeation mechanism in a membrane means obtaining answers to at least the following five questions, of which the first two arise in any membrane and the last two arise only in very thin membranes.

1. Do cations and anions have the same or different permeation mechanisms and routes through the membrane? In other words, is the membrane a mosaic as regards ion permeation?

2. Are the membrane groups or sites controlling ion permeation (i.e., the nearest neighbors to permeating ions) fixed or mobile within the membrane?

3. Do the permeation-controlling sites bear a net charge? If membrane groups with net charge are present, as in the various types of ion-exchange membranes, then in a thick membrane the electroneutrality condition requires that there be a net excess of oppositely charged mobile ions from the bathing solution ("counter-ions") over like-charged mobile ions ("co-ions"), to balance these membrane charges. If the answer is no, as in "neutral" membranes (e.g., membranes with dipolar sites), then the membrane must contain nearly identical numbers of positive and negative mobile ions if it is thick.

4. In a very thin membrane, one must ask if microscopic electroneutrality is still obeyed within the membrane. If the membrane is sufficiently thin and the concentration of ions within it is sufficiently low, then the Debye length (the mean distance between cations and anions)[1] may become comparable to or greater than the membrane thickness. Thus, the membrane (inclusive of the ions in it) could bear a small net charge, which would be balanced by ions immediately outside the membrane (*see* Barry & Diamond, 1971, pp. 310–319 – henceforth referred to as paper II – for further discussion). For instance, thin lipid membranes containing ion-complexing antibiotics such as valinomycin and monaction apparently have a net charge (Ciani, Eisenman & Szabo, 1969). This breakdown of the electroneutrality condition results in some of the differences between ion exchangers and dipolar sites becoming obscured in thin membranes.

5. In a sufficiently thick membrane, the resistances associated with crossing the two membrane-solution interfaces will become negligible compared to the diffusional resistance of the membrane interior, but the inter-

1 For a precise definition of the Debye length in membranes which violate electroneutrality, *see* paper II, Eq. (72), or Ciani, Eisenman & Szabo, 1969, Eq. (43).

facial resistance may become increasingly significant as membrane thickness is reduced and the diffusion pathway is shortened. One is thus faced with the practical question of deciding if biological membranes are thin enough for interfacial effects to be detectable. In the present analysis, we shall interpret the measured properties of the gallbladder in terms of permeation models which consider the interfacial resistance to be negligible. This assumption seems reasonable, since the water permeability of artificial thin lipid membranes is that expected of a 50 Å layer of lipid with the diffusional resistance of bulk hexane (Finkelstein & Cass, 1968), since cyclic antibiotics which solubilize ions in bulk lipid solvents lower the electrical resistance of artificial thin lipid membranes and of some biological membranes, and since the properties of such antibiotic-treated membranes agree in considerable detail with those predicted on the assumption that the membrane-interior resistance is dominant (Szabo, Eisenman & Ciani, 1969). No properties of ion permeation in the gallbladder have been found which appear inconsistent with this assumption. In particular, the form of the current-voltage relation for the gallbladder (p. 354) suggests, although it does not prove, that interfacial effects are not rate-limiting in the present experiments and that the rate-controlling membrane in the gallbladder is relatively thick.

Types of Experiments to Test Ion Permeation Mechanisms

We have used the following six types of "black-box" measurements as evidence that might answer these questions:

1. Current-Voltage Curves. Measurement of the p.d. (potential difference) across the membrane as a function of applied current in single-salt solutions.

2. Conductance-Concentration Relations. Measurements of membrane conductance (limiting slopes of current-voltage curves) as a function of total salt concentration.

3. Dilution Potentials. The p.d. in the absence of applied current when the membrane separates solutions of the same salt at different concentrations, such as 150 mM NaCl vs. 75 mM NaCl.

4. Biionic Potentials. The p.d. in the absence of applied current when the membrane separates solutions of two different salts sharing a common anion (or cation) at the same total salt concentration, such as 150 mM NaCl vs. 150 mM KCl.

5. Cation Selectivity Sequences. Comparison of the relative permeabilities of the five alkali cations as judged from conductances, dilution poten-

tials, and biionic potentials, and from the Ca^{++} competition effect (p. 348), which appears to measure equilibrium selectivity.

6. Tracer Fluxes. Comparison of partial ionic conductances based on tracer fluxes and on electrical measurements. One-way fluxes of Na^+, K^+, and Cl^- or Br^- in gallbladder have been previously determined by Diamond (1962) and by Wheeler (1963) with essentially the same results and have therefore not been repeated in the present study.

Ion Permeation Models

There were five different simple models of ion permeation which at various early stages in this study showed some promise of explaining the gallbladder results and which are therefore frequently cited in the text for confrontation with the results. The first four of these models are based on thick membranes; the fifth applies to a membrane thin enough to violate microscopic electroneutrality. These models are given below.

1. Single-Channel Fixed-Site Ion Exchanger. Ion-exchange membranes containing a single type of fixed site are well understood theoretically and experimentally as a result of the studies of Teorell (1953), Meyer and Sievers (1936), and Conti and Eisenman (1965). When bathing-solution salt concentrations are well below the concentration of fixed sites in the membrane, co-ions are virtually absent from the membrane ("Donnan exclusion"), and the concentration of counter-ions approximately equals the site concentration. Thus, in dilute bathing solutions the membrane is permeable only to counter-ions, conductance is nearly independent of bathing solution concentration, and dilution potentials plotted against the logarithm of the activity ratio give linear Nernst slopes (58.8 mV per 10-fold activity difference at 23 °C). In bathing solutions whose salt concentrations approach and exceed the membrane fixed-charge density, the co-ion concentration in the membrane begins to increase, conductance rises, and dilution potential slopes decrease towards the values for free-solution junction potentials with increasing bathing-solution salt concentration (Fig. 1).

2. Fixed-Site Ion-Exchange Mosaic. Mosaic ion exchangers with parallel regions of fixed negative sites and fixed positive sites have been discussed by Conti and Eisenman (1965). For bathing-solution salt concentrations well below the site concentrations of either channel, such membranes are permeable to both cations and anions, and dilution potentials give linear slopes but with values less than 58.8 mV. Conductance is still independent

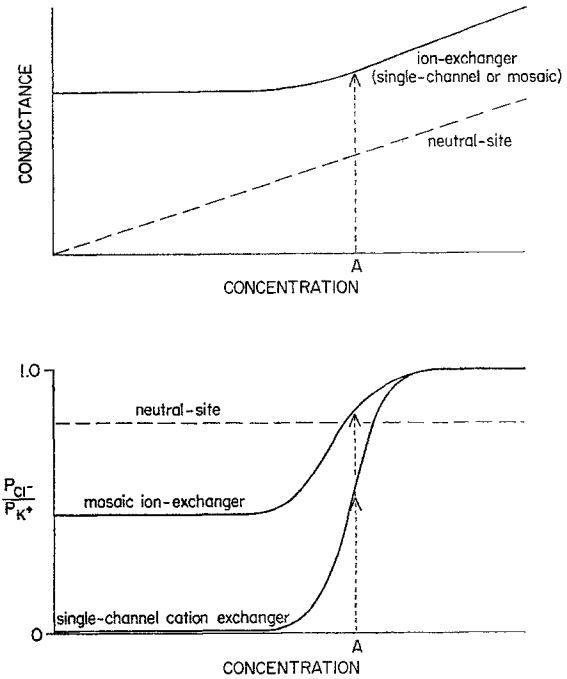


Fig. 1. *Above:* predicted conductance-concentration curves for three ion permeation models. In a neutral-site membrane, the relation is linear. In a single-channel cation exchanger (Teorell, 1953) or mosaic ion exchanger, the conductance is initially independent of concentration and begins to increase towards a linear asymptote only as the membrane fixed-charge concentration (*A* on the abscissa, and dotted arrow) is approached. *Below:* predicted dependence of the anion/cation permeability ratio (e.g., P_{Cl}/P_K) on concentration. In a neutral-site membrane both cations and anions may have finite permeabilities, and the ratio is independent of concentration. In a single-channel cation exchanger, the anion permeability is negligible at low concentrations ($P_{Cl}/P_K \sim 0$), but the ratio asymptotically approaches the free-solution mobility ratio (1.0 in the case of KCl) as the membrane fixed-charge concentration (*A* on the abscissa, dotted arrow) is approached. In a mosaic ion exchanger, the ratio similarly approaches the free-solution mobility ratio at high concentrations but has a non-zero value at low concentrations. All the curves in this figure are formulated for a membrane thick enough that microscopic electroneutrality is obeyed. The concentration scales and the conductance scale are logarithmic

of bathing solution concentration until bathing salt concentrations become comparable with site concentrations, at which point conductance begins to increase and dilution potentials begin to approach the values of free-solution junction potentials (Fig. 1).

3. Mobile-Site Ion Exchangers. Membranes containing charged sites which are mobile within the membrane may differ from fixed-site ion exchangers in two respects (Conti & Eisenman, 1966; Sandblom, Eisenman &

Walker, 1967). Current-voltage curves in symmetrical solutions of single salts are no longer linear, as in fixed-ion exchangers, but instead a saturating current is reached at high voltages when most of the sites are piled up at one side of the membrane. If the site and the counter-ion are firmly associated, shuttling of the neutral ion-plus-site complex across the membrane has no direct electrical manifestations, so that partial ionic conductances estimated from tracer fluxes exceed conductances estimated electrically ("exchange diffusion").

4. Fixed Neutral Sites. Even though a membrane lacks sites with net charge, it may still discriminate between cations and anions, because membrane components or else specific sites will in general contain polar groups such as dipoles. Depending on whether the route of ion permeation passes nearer the negative or the positive ends of the dipoles, the membrane will be preferentially permeable to cations or to anions. Two examples of neutral membranes exhibiting ion selectivity may be cited: a thick membrane composed of a layer of nitrobenzene interposed between two aqueous phases is much more permeable to Cs^+ than to F^- (Eisenman, Sandblom & Walker, 1967); and thin lipid membranes treated with the "pore-forming" polyene antibiotics nystatin and amphotericin B are anion-selective (Finkelstein & Cass, 1968; Cass, Finkelstein & Krespi, 1970). The theories of models in which fixed neutral sites are embedded in either a thick membrane or a thin membrane are derived in the preceding paper (paper II). In dilute solutions, the dilution potentials for such a membrane model are expected to give linear slopes which in general differ from 58 mV, as also true for a mosaic ion exchanger. However, conductance is a linear function of bathing solution concentrations, in contrast to the case of a mosaic ion exchanger (Fig. 1).

5. Thin Membranes with Mobile Neutral Sites. This model differs from the previous one in that the dipolar sites are mobile and in that the membrane is thin enough to deviate from microscopic electroneutrality. Thin lipid membranes containing neutral cation-sequestering antibiotics such as valinomycin or monactin appear to exemplify this model (Ciani *et al.*, 1969; Szabo *et al.*, 1969). In dilute solutions, anion permeability is negligible, and conductance (due to cations) is a linear function of bathing solution concentrations. Provided that the rates of formation and dissociation of the ion-site complex at the membrane surfaces are sufficiently rapid, conductance ratios or permeability ratios for different cations equal equilibrium-constant ratios, since complexes of a given site with any cation have essentially the same mobility. This is in contrast to the expectation based on

fixed neutral sites (model number 4), where different cations will in genera have different mobilities.

Permutations of these "basic" models are also possible. For instance one may have a mosaic of mobile ion-exchange sites for anions and fixed neutral sites for cations; and any of the first four mechanisms, which were stated in terms of thick membranes, may also operate in thin membranes with appropriate modification. Our experiments suggest that the permeation mechanism in the gallbladder represents one of these five "basic" mechanisms, modified by the presence of a shunt.

Materials and Methods

Most experimental techniques were discussed in paper I. Gallbladder conductance was measured as described previously (Wright & Diamond, 1968), i.e., by observing the transepithelial potential difference when a direct current (usually 100 μamp) was passed across the tissue. Briefly, the technique was to clamp a flat sheet of gallbladder across a window of area 1.13 cm^2 between two lucite chambers, each with a volume of 10 ml. The p.d. was recorded with two agar salt bridges placed 1.3 mm from each side of the tissue, while current was passed across the tissue by means of Ag/AgCl electrodes and agar salt bridges and was measured on a Keithley 600 A electrometer. The "edge" conductance associated with the damaged ring of tissue clamped between the chambers must be negligible compared to the conductance of the rest of the tissue, since the same value of conductance per unit area was obtained whether the chambers had an exposed area of 1.13 cm^2 or 2.6 mm^2. The experimental solutions used for conductance experiments consisted of 150 mM alkali halide, 0.25 mM $CaCl_2$, and 1.5 mM Tris buffer at pH 7.4. All conductance values were corrected for the conductance of the saline between the tips of the salt bridges used to monitor the p.d. As shown previously (Wright & Diamond, 1968), at least 94% of the gallbladder resistance is provided by the epithelium. In most of the present experiments, the outer serosal muscle layers were dissected from the gallbladder in order to minimize time-dependent conductance changes due to the contraction of the muscle in KCl, RbCl, or CsCl solutions. These changes took the form of a slow continuous decrease in gallbladder conductance without change in the anion : cation permeability ratio and were probably due to occlusion of microscopic folds of epithelium. Returning the gallbladder from KCl, RbCl, or CsCl to NaCl or LiCl solutions rapidly brought the conductance back to its initial value in these latter two salts, which do not cause contraction of the muscle. In some experiments, the conductance decrease in RbCl or CsCl began so soon after transfer of the gallbladder to these salts that the conductance change owing to ionic composition could not be determined.

Determination of current-voltage relations was carried out in chambers essentially the same as those used for conductance measurements, except that the area of gallbladder between the chambers was reduced to 2.6 mm^2 in order that much higher current densities could be used without having to require too much current from the Ag/AgCl plate electrodes and current source. All voltage measurements were made within the first 20 msec of a constant-current pulse in an attempt to eliminate voltage creep caused by the transport-number effect (Barry & Hope, 1969*a*, *b*; Wedner & Diamond, 1969), which begins to cause a detectable progressive increase in the voltage across the gallbladder after about a half-second. The rise in voltage after onset of a constant-current pulse approximated a single exponential, with a time constant of 0.21 ± 0.03 msec

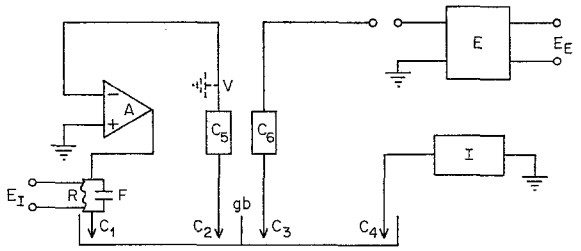


Fig. 2. Schematic diagram of the circuit used to measure the current-voltage relation of the gallbladder (indicated by *gb*). *C1* and *C4* refer to Ag/AgCl current-passing electrodes, *C2* and *C3* to potential-measuring electrodes connected to calomel electrodes (*C5* and *C6*). The point *V* is maintained at virtual earth by means of the operational amplifier *A*. *E* refers to the Keithley 604 electrometer, whose output E_E is connected to the voltage trace of the dual-beam oscilloscope; *I* is the constant-current generator. *R* is the current-recording resistor of 1 kΩ, whose potential drop E_I is connected to the current trace of the oscilloscope. *F* refers to the 0.01 μfarad capacitor used to filter out high-frequency noise and to stabilize the operational amplifier

(average value ±S.E.M. in three gallbladders). The capacitance calculated from this time constant and from the gallbladder resistance in these experiments was 10±3 μfarad/cm² of nominal gallbladder surface area (i.e., assuming the gallbladder to be a flat sheet). This value is in reasonable agreement with expectations based on a typical capacitance of 1 μfarad/cm² cell membrane, multiplied by the factor of 14 by which the actual mucosal surface area is increased over the nominal value due to epithelial folding and microvilli (Smulders, 1970).

To make these rapid voltage measurements at constant current, a pulse circuit (Devices Digitimer, type 3290) was used to trigger a stimulator (Devices Isolated Stimulator Mark IV), which in turn triggered a pulse from a constant-current generator. The power supply for the current generator was taken from a Heathkit variable-voltage regulated power supply. The current was recorded as the voltage drop across a 1.00± 0.01 kΩ resistor. The p.d. across the gallbladder was measured using two calomel half-cells. [In practice, the p.d. was measured between one calomel electrode and earth with a fast-response Keithley 604 electrometer set at unity gain; the second calomel electrode was maintained at virtual earth through an operational amplifier (*see* Fig. 2). Similarly, the current generator passed current between the right-hand current electrode and earth. Such an arrangement was used to reduce the magnitude of the large absolute voltages above earth that would have existed at the electrometer inputs if one of the inputs had not been earthed.] Both the current and voltage signals were connected to the inputs of a 502 A dual-beam Tektronix oscilloscope, and the actual measurements were made from Polaroid photographs of the signal traces. All voltage measurements were corrected for the voltage drop in the saline between the tips of the salt bridges used to monitor the p.d.

Effects of the relatively impermeant cation Ca^{++} on 2:1 dilution potentials were determined by adding an isotonic $CaCl_2$ solution to the mucosal bathing solution of 75 mM alkali halide plus mannitol, in proportions to increase (Ca^{++}) to 5.25 mM from the usual value of 0.25 mM. The junction potentials calculated from the modified Planck-Henderson equation [paper I, Eq. (1)] to correct measured p.d.'s for these high-Ca^{++} 75 mM-alkali halide solutions against a 150 mM agar bridge of the same salt were 5.4 mV

for LiCl, 3.0 mV for NaCl, −0.4 mV for KCl, −0.8 mV for RbCl, and −0.8 mV fo CsCl.

Although p.d.'s and conductances in the gallbladder are relatively stable, gradua changes in their values become apparent in experiments lasting several hours, presum ably owing to deterioration of the tissue and development of a free-solution shunt For all alkali halides, dilution potentials decline with time (paper IV), implying a slov decrease in the cation/anion permeability ratio. Hence all experimental measurement utilized "bracketed" experimental designs in which a measurement in one salt or a one concentration was both preceded and followed by measurements with a differen salt or a different concentration, so that each preparation served as its own contro (*cf.* Figs. 5, 7 & 9).

P.d.'s were measured in either of two ways described in paper I: (1) by using th salt-bridge arrangements chosen to obtain well-defined time-independent junctio potentials and then correcting for the values of junction potentials listed in the las column of Table 2 of paper I; or (2) by using Ag/AgCl electrodes and correcting for th difference in electrode potentials calculated from the Guggenheim assumption. A shown previously (paper I, Fig. 5), results from these two recording arrangement agree closely. All p.d.'s are given as the potential of the serosal solution with respec to that of the mucosal solution. The p.d. in the absence of ion concentration gradient was always less than 1 mV. All errors are reported as standard errors of the mean

Results

Variation of Conductance with Time

The initial time dependence of gallbladder conductance in NaCl is illustrated in Fig. 3. Within the first few minutes of setting up a preparation,

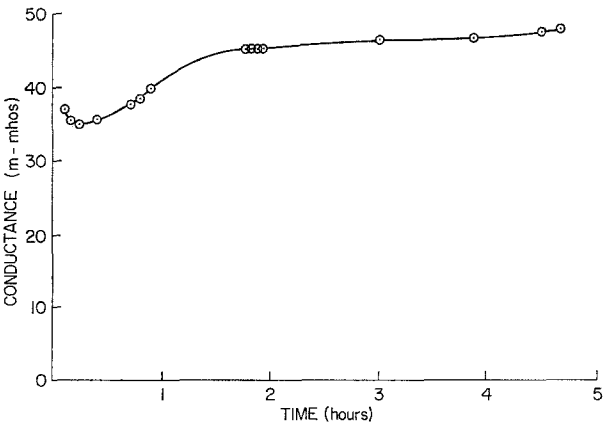


Fig. 3. The conductance of gallbladder epithelium in 150 mM NaCl solution (ordinate) as a function of time after dissection (abscissa). See test for experimental details. Because of the initial transient changes, effects of solution composition on conductance were determined about 100 min after dissection, at a time when conductance has become stable. The gallbladder area used in the experiments of this figure and Figs. 4 and 5 was 1.13 cm^2

there was a slight decline in conductance, followed by an increase until a steady state was achieved after about 60 to 90 min. The average value ±S.E.M. of this steady-state conductance in 150 mM NaCl solution was 36 ± 1 mmho/cm^2 (18 gallbladders) at 120 min after dissection. To eliminate this initial time dependence (probably due to the development of a free-solution shunt: *see* paper IV, Fig. 6) as a variable when studying the effect of ionic composition, the conductance in 150 mM NaCl solution was always determined as a control immediately before and after measuring the conductance in a different salt.

Conductance-Concentration Relations

The dependence of gallbladder conductance on salt concentration in symmetrical solutions was studied in NaCl and in LiCl. A gallbladder was initially left in 150 mM NaCl or 150 mM LiCl until the conductance had achieved a steady value. The salt concentration in the mucosal and serosal solutions was then lowered symmetrically and simultaneously by isoosmotic replacement with mannitol, and the conductance was recorded once a new steady value had been achieved and the transient p.d. owing to the asymmetry of the unstirred layers (paper I, p. 117) had disappeared. Finally, conductance was measured again at 150 mM before proceeding to a new salt concentration. As illustrated in Fig. 4 for one gallbladder in NaCl and another in LiCl, conductance increases approximately linearly with con-

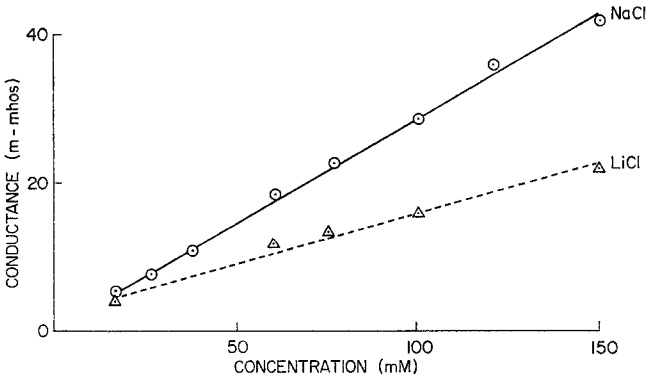


Fig. 4. Dependence of gallbladder conductance (ordinate) on bathing-solution salt concentration (abscissa). In the experiment of the upper curve, conductance was initially measured with 150 mM NaCl both as the mucosal and the serosal bathing solution, and then as the salt concentration in both bathing solutions was lowered step-wise to 16 mM by isoosmotic replacement with mannitol. The experiment of the lower curve was similar except for being carried out in LiCl. Note that the relation between conductance and concentration is approximately linear and that conductance is lower in LiCl than in NaCl

centration in either salt. The same result was obtained in five other experiments, which showed in addition that linearity was preserved up to concentrations of 400 mM. A similar linear conductance-concentration relation holds for rabbit intestine (Schultz, Curran & Wright, 1967) and frog choroid plexus (Wright, *unpublished observation*).

The Cation Selectivity Sequence Deduced from Conductance

The procedure for studying conductance differences between 150 mM solutions of different alkali cations is illustrated in Fig. 5. In this experment, the gallbladder was initially kept for 100 min in 150 mM NaCl solution. When the mucosal and serosal solutions were changed symmetrically to 150 mM LiCl, the conductance decreased from about 40 mmho to 22.5 mmho and stabilized at 24.5 mmho. In a mixture of 75 mM LiCl–75 mM NaCl, the conductance rose to a value intermediate between that in 150 mM NaCl and that in 150 mM LiCl. Finally, when 150 mM NaCl was restored, the conductance returned to near its original value. In a total of eight similar experiments, replacement of 150 mM NaCl with 150 mM LiCl was found to reduce the gallbladder conductance on the average by 40%. The Table summarizes the results of all 19 experiments in which conductance in a 150 mM solution of an alkali halide was determined relative to the conductance in 150 mM NaCl. The apparent conductance sequence is $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$. As will be discussed in the next paper (paper IV,

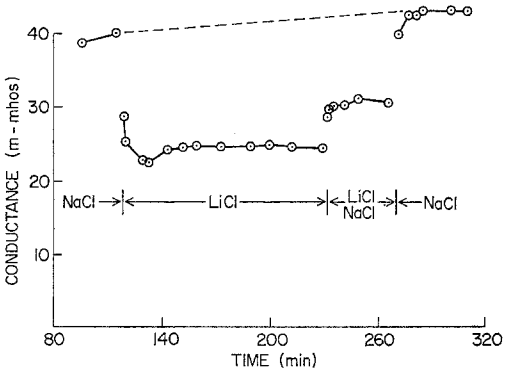


Fig. 5. Comparison of gallbladder conductance in NaCl and LiCl. Conductance was measured successively in 150 mM NaCl, 150 mM LiCl, a 75 mM LiCl–75 mM NaCl mixture, and finally in 150 mM NaCl again. During each measurement, the mucosal and serosal solutions were identical in composition. After each change of solutions, a few minutes was required for conductance to reach a new steady-state value. Note that conductance in LiCl is about 60% of that in NaCl

Table. *Relative conductance of gallbladder epithelium in different alkali chloride solutions*

Salt	Relative conductance[a]
KCl	1.5±0.1 (9)
RbCl	1.2±0.1 (3)
NaCl	1.0
CsCl	0.8±0.1 (5)
LiCl	0.6±0.1 (8)

[a] Average value ± standard error of the mean (number of experiments in parentheses) of gallbladder conductance in a solution of the given salt at 150 mM, divided by the conductance in 150 mM NaCl solution as measured immediately before and after the measurement in the test salt. The differences in conductance among different alkali chlorides are quantitatively small compared to the differences in many other biological membranes; the possible significance of this fact is considered in the following paper (paper IV). As discussed in the text, it is uncertain after correction for shunt conductance whether CsCl conductance is actually greater than the LiCl value or vice versa.

p. 369), some of the measured conductance is in a shunt, where ion mobilities are in free-solution ratios. Correcting the measured total conductance for shunt conductance lowers Cs^+ membrane conductance toward the value for Li^+, and it is uncertain what the actual order of the membrane conductance of these two ions is. Hence, the sequence in native gallbladder membrane can be stated only as $K^+ > Rb^+ > Na^+ > Cs^+, Li^+$.

The Current-Voltage Relation

Instantaneous current-voltage characteristics were measured in five gallbladders in symmetrical 150 mM NaCl solutions for currents up to 22 mamp/cm^2. The I–V curves were linear and symmetrical within an accuracy of ±2%, even up to 800 mV (Fig. 6a). In six experiments on three gallbladders, I–V curves were determined in asymmetrical NaCl solutions (30 mM NaCl vs. 150 mM NaCl) and were still linear and symmetrical up to the highest voltage measured, 750 mV (Fig. 6b). These high voltages appeared to have no damaging effect on the permeability characteristics of the gallbladder, as judged from the position of subsequent points on the I–V curve and from the values of subsequent 2:1 dilution potentials. Similarly, the current-voltage relation in another epithelium, frog choroid plexus, is linear in both symmetrical and asymmetrical solutions up to at

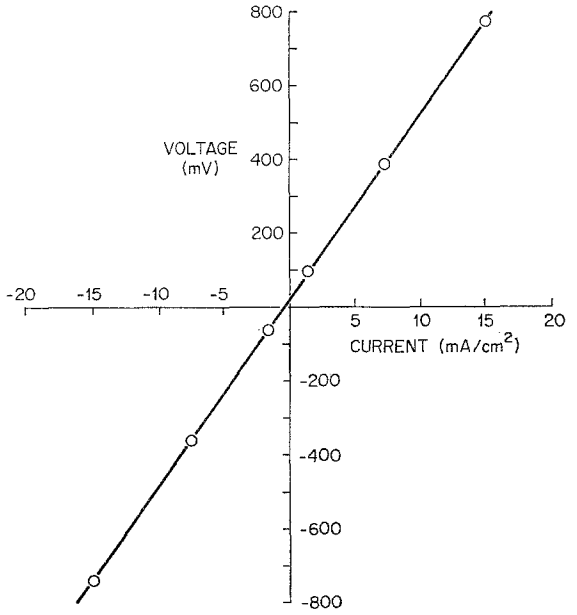
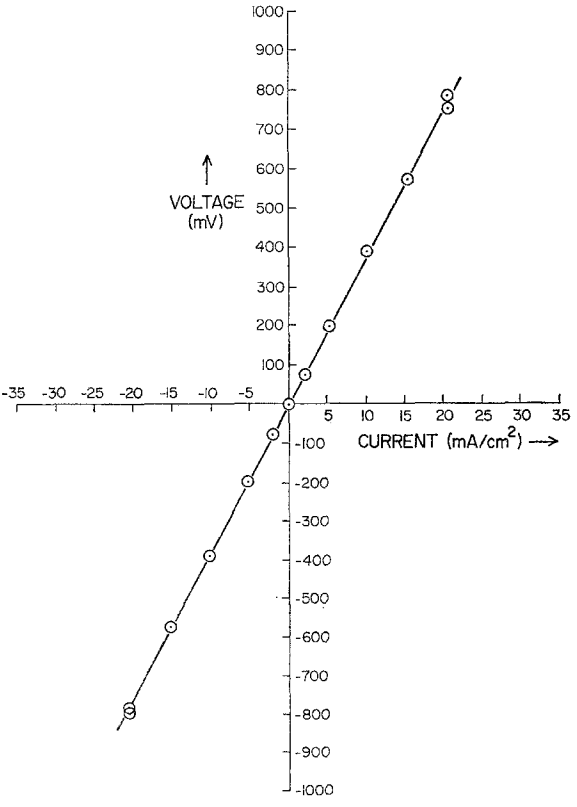

800
600
VOLTAGE
(mV)
400
200
-20
-15
-10
-5
5
10
15
20
CURRENT (mA/cm^2)
-200
-400
-600
-800

Fig. 6a


1000
900
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700
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VOLTAGE
(mV)
500
400
300
200
100
-35
-30
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-20
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-5
5
10
15
20
25
30
35
CURRENT (mA/cm^2) ⟶
-100
-200
-300
-400
-500
-600
-700
-800
-900
-1000

Fig. 6b

least several hundred mV (Wright, *unpublished observation*). The significance of these results is discussed on pp. 352–354 [2].

The Anion: Cation Permeability Ratio as a Function of Concentration

Two experiments to determine the dependence of permselectivity (as measured by 2:1 dilution potentials) on salt concentration were carried out, using RbCl solutions in order to minimize junction potential corrections. In combination with the conductance-concentration relation described above, these measurements provide a test of the possibility that the gallbladder behaves in the manner of a macroscopic hydratable ion-exchange membrane (*see* Teorell, 1953) toward permeating cations. The mucosal and serosal solutions were initially 50 mM RbCl (this and the remaining solutions in this experiment were kept isoosmotic with 400 mM RbCl by incorporating appropriate concentrations of mannitol), and 2:1 dilution potentials were measured by lowering the mucosal concentration to 25 mM RbCl or by raising it to 100 mM. Then the mucosal and serosal solutions were changed to 200 mM RbCl, and the dilution potentials resulting from changing the mucosal concentration to 100 mM or to 400 mM were

2 In some gallbladders there were initially transients in the voltage traces for current densities greater than 5 mamp/cm^2 in the direction making the serosa positive. These transients gradually decreased and eventually disappeared with time after dissection, with repetitive passage of current pulses, or after several changes of the bathing solutions. The form of the transients was that the voltage decreased by up to 20% after reaching a maximum within 2 msec of current pulse initiation and then increased again back to the steady-state level within about 10 msec. Associated with the transients, and disappearing as they disappeared, was a slight deviation ($\leqq 10\%$) from linearity in the I–V curve in the sublinear direction at higher current densities (22 mamp/cm^2). In each case, there was no change in the 2:1 dilution potential as the transient disappeared, implying that the transient was not directly related to the permeation mechanism. The phenomenon probably arises from the transport-number effect, i.e., fast current-induced concentration changes in an unstirred layer (Wedner & Diamond, 1969; Barry & Hope, 1969*a*, *b*).

Fig. 6. a) The instantaneous current-voltage relation for a gallbladder bathed in symmetrical 150 mM NaCl solutions. The voltage drop across the gallbladder produced by a constant current was measured within the first 20 msec of onset of the current pulse. The gallbladder area use in this experiment and in the experiment of Fig. 6b was 2.6 mm^2. Note that the relation is linear. b) The instantaneous current-voltage relation for a gallbladder bathed in asymmetrical salt solutions. The serosal solution contained 150 mM NaCl; the mucosal solution contained 30 mM NaCl plus mannitol to maintain isotonicity of the solution. The voltage drop across the gallbladder produced by a constant current was measured within the first 20 msec of onset of the current pulse. The resistance of the same gallbladder in symmetrical 150 mM NaCl solutions was 55% lower. Different gallbladders were used for the experiments illustrated in Figs. 6a and b

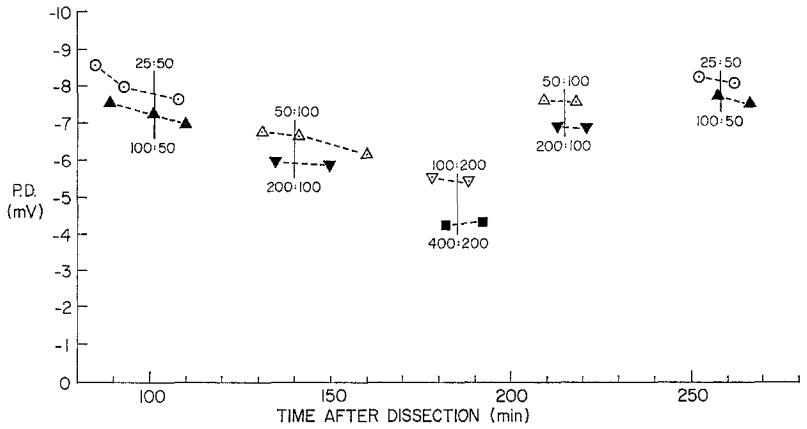

Fig. 7. An experiment designed to measure the anion/cation permeability ratio of gallbladder epithelium as a function of ionic strength. Dilution potentials resulting from 2:1 concentration gradients of RbCl were measured at four different absolute concentration levels: 50 vs. 25 mM (⊙), 100 vs. 50 (△ and ▲), 200 vs. 100 (▽ and ▼), and 400 vs. 200 (■). The solution with the higher salt concentration was in some cases the mucosal solution (closed symbols), and in other cases the serosal solution (open symbols), but it is apparent that the direction of the gradient has no effect on the magnitude of the dilution potential (except for changing the sign). The points with closed symbols have been plotted with reversed sign to facilitate comparison. All solutions were kept isoosmotic with 400 mM RbCl by incorporation of mannitol. Interpolated values of pairs of dilution potentials were compared at the times indicated by the vertical lines, and the P_{Cl}/P_{Rb} ratios calculated from these dilution potentials by means of the Goldman-Hodgkin-Katz equation are replotted in the lower half of Fig. 8

measured. Finally, both bathing solutions were changed back to 50 mM RbCl, and the 50:25 and 50:100 dilution potentials were again determined. Fig. 7 illustrates the results of one such experiment.

For quantitative analysis of these results, the permeability ratio P_{Cl}/P_{Rb} was calculated from the measured p.d.'s by means of the Goldman-Hodgkin-Katz ("constant-field") equation [*see* paper IV, Eq. (1)][3]. Permeability ratios for pairs of gradients were calculated from measured or interpolated p.d.'s at the same time and were then all normalized to the same time to permit comparisons among all four gradients. Fig. 8 shows the resulting calculated dependence of P_{Cl}/P_{Rb} on the average RbCl concentration for

3 As shown in the following paper (paper IV, pp. 362 and 389), this equation gives a good but not perfect fit to p.d.'s measured in the gallbladder. Permeability ratios have also been calculated for the present experiment from an equation [paper II, Eq. (53)] which will be shown in the following paper to fit measured p.d.'s more exactly and which we think may provide a physically realistic description of permeation through the gallbladder. Analysis of the results of Fig. 7 by either equation yields quantitatively similar permeability ratios and qualitatively identical conclusions.

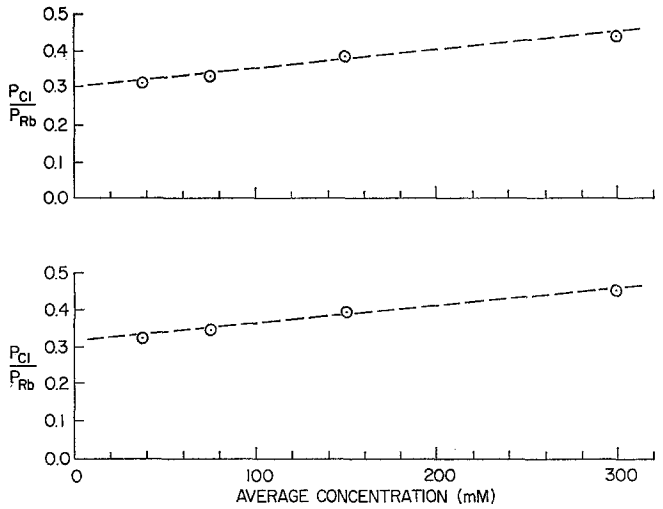

Fig. 8. Anion/cation permeability ratios in the gallbladder calculated from the Goldman-Hodgkin-Katz equation [paper IV, Eq. (1)], as a function of absolute salt concentration (ionic strength). Dilution potentials resulting from 2:1 gradients of RbCl were measured at four different absolute concentration levels, as in the experiment of Fig. 7. To correct for the irregular small variation of p.d.'s with time over the course of an experiment (as in Fig. 7), the average value of $\alpha = P_{Cl}/P_{Rb}$ at a 50:25 mM gradient was used to normalize the values at other gradients, by calculating the average ratio of the permeability ratios $\frac{\alpha(100:50)}{\alpha(50:25)}$ at a given time (*see* Fig. 7 for interpolation procedure) and multiplying by the average value of $\alpha(50:25)$, then calculating the average ratio $\frac{\alpha(200:100)}{\alpha(100:50)}$ and multiplying by the normalized $\alpha(100:50)$, and finally calculating the average ratio $\frac{\alpha(400:200)}{\alpha(200:100)}$ and multiplying by the normalized $\alpha(200:100)$. The abscissa gives the average of the two salt concentrations used to produce a given gradient. The lower curve is derived from the gallbladder of Fig. 7, whereas the upper curve is derived from another gall-bladder. Note that P_{Cl}/P_{Rb} increases only slightly (by 0.05/100 mM) over the average concentration range 37.5 to 300 mM

the two gallbladders. The ratio shows only slight concentration dependence over the range 37.5 to 300 mM, increasing by 0.053 and by 0.051 per 100 mM in each gallbladder, respectively, from a value of about 0.3 in dilute solutions. This implies a slight decrease in cation conductance or a slight increase in anion conductance, or both, with increasing concentration. However, the magnitude of these conductance changes would be insufficient to produce significant nonlinearity in conductance-concentration curves, in agreement with the fact that no nonlinearity was detected (Fig. 4).

In two similar experiments in KCl solutions, the ratio P_{Cl}/P_K showed no significant concentration dependence when compared at gradients of 25 vs. 50 mM, 50 vs. 100 mM, and 100 vs. 200 mM.

The Cation Selectivity Sequence Deduced from the Ca^{++} Competition Effect

Ca^{++} and other polyvalent cations depress NaCl dilution potentials and increase the ratio P_{Cl}/P_{Na} by depressing Na^+ conductance while increasing Cl^- conductance (Diamond & Harrison, 1966; Wright & Diamond, 1968). The effect probably involves a competition between Ca^{++} and Na^+ for cation-binding sites (gallbladder permeability to Ca^{++} is too low for Ca^{++} itself to contribute directly to the p.d., since variation in (Ca^{++}) on one side of the membrane in the absence of other gradients causes no p.d. shift). Hence the effect of Ca^{++} on dilution potentials of other alkali chloride salts was measured, in order to be able to rank the alkali cations in their relative ability to compete with Ca^{++} and hence presumably in their equilibrium affinities for the cation-binding site.

Fig. 9 illustrates an experiment in which the effect of Ca^{++} on NaCl and on KCl dilution potentials was compared. Dilution potentials resulting from a 2:1 concentration gradient were measured in NaCl solution, then in KCl, and finally in NaCl again. In each solution, several dilution poten-

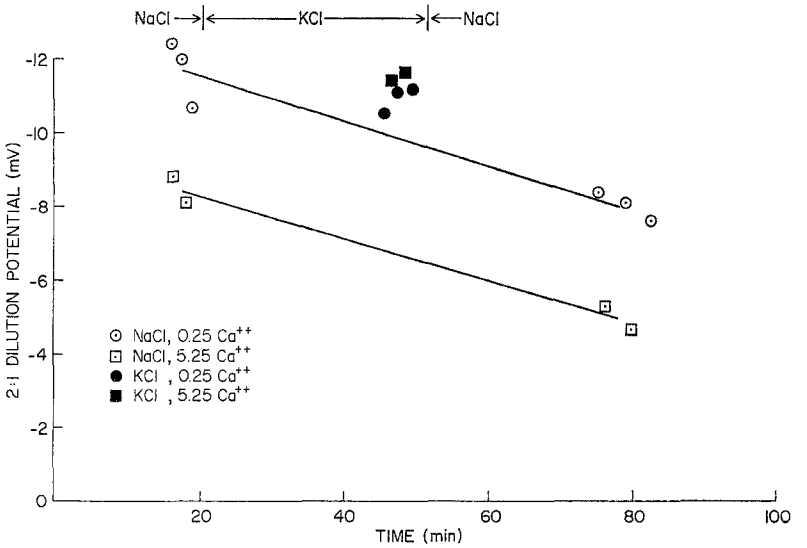

Fig. 9. Comparison of the effect of Ca^{++} on 2:1 dilution potentials of NaCl and of KCl. Initially the gallbladder was in 150 mM NaCl, and the dilution potentials resulting from lowering mucosal NaCl to 75 mM by isoosmotic replacement with mannitol were measured when the mucosal [Ca^{++}] was either 0.25 (⊙) or 5.25 (⊡) mM. The same comparison was then carried out for 2:1 dilution potentials in KCl (0.25 mM Ca^{++} ●, 5.25 mM Ca^{++} ■), and finally in NaCl again. Note that Ca^{++} depresses NaCl dilution dotentials by 30% but scarcely affects KCl dilution potentials, and that at a given [Ca^{++}] p.d.'s are higher in KCl than in NaCl

tials were determined in the presence of the normal [Ca^{++}] of 0.25 mM and after [Ca^{++}] of the mucosal solution had been raised to 5.25 mM (similar results were obtained when [Ca^{++}] in both bathing solutions was raised symmetrically). In accordance with the earlier observations (Wright & Diamond, 1968), the increased [Ca^{++}] reduced NaCl dilution potentials by about 32% in this gallbladder, implying an increase in P_{Cl}/P_{Na} (as calculated by the Goldman-Hodgkin-Katz equation) from 0.22 to 0.46, i.e., by a factor of 2.1. In contrast, the effect of [Ca^{++}] on KCl dilution potentials was very small (Fig. 9) and constituted in fact a slight but insignificant increase. For all gallbladders tested, addition of 5 mM [Ca^{++}] increased P_{Cl}/P_{Na} on the average by 2.0 ± 0.1 ($n=33$) times (from 0.31 ± 0.02 on the average to 0.58 ± 0.03 on the average), but increased P_{Cl}/P_K by only 1.1 ± 0.1 ($n=7$) times (from 0.17 ± 0.01 to 0.21 ± 0.01). KCl dilution potentials were also found to be insensitive to 5 mM mucosal concentrations of the three other alkaline-earth cations, Ba^{++}, Sr^{++}, and Mg^{++}, of which the first two depress NaCl dilution potentials (Wright & Diamond, 1968).

Thirteen experiments similar to that illustrated in Fig. 9 were carried out to determine the effects of Ca^{++} on other pairs of alkali chlorides. In two gallbladders where the effect of Ca^{++} on dilution potentials in NaCl and in RbCl was compared, the effect in NaCl was greater in both cases. In one comparison of KCl and RbCl, the effect in RbCl was greater. In four comparisons of CsCl and LiCl, the effects were not distinguishable in three cases, greater for CsCl in the fourth. In four comparisons of NaCl and LiCl, the effects were not distinguishable in three cases, greater for NaCl in the fourth. In two comparisons of NaCl and CsCl, the effects were not distinguishable in one case, greater for NaCl in the other. Combining these comparisons of cation pairs yields the following sequence for decreasing effects of Ca^{++} on dilution potentials: $Na^+ \sim Cs^+ \sim Li^+ > Rb^+ > K^+$. The same sequence is obtained if one calculates for all experiments the average factor by which Ca^{++} increases the Cl^-/cation permeability ratio: NaCl (2.0 ± 0.1, $n=33$) ~ CsCl (2.0 ± 0.1, $n=18$) ~ LiCl (1.8 ± 0.1, $n=23$) > RbCl (1.4 ± 0.1, $n=7$) > KCl (1.1 ± 0.1, $n=7$).

A factor which might have complicated the interpretation of the Ca^{++} effect is that Cl^-/cation permeability ratios in the gallbladder tend to increase as a function of time because of development of a shunt (paper IV, p. 369), and that the factor by which Ca^{++} increases the Cl^-/cation permeability ratio tends to decrease with time. However, this complication may be eliminated by the alternative method of comparing Ca^{++} effects on different cations illustrated in Fig. 10, which plots for each cation the Cl^-/cation permeability ratio at 0.25 mM Ca^{++} against the ratio at 5.25 mM

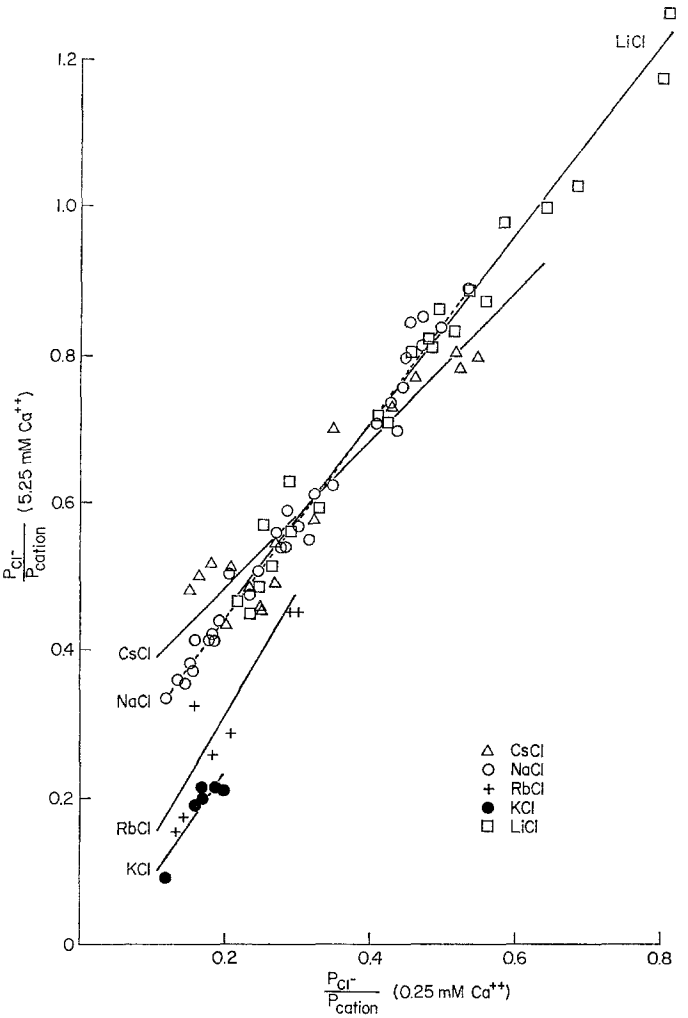

Fig. 10. The ratio P_{Cl}/P_{cation} in the presence of 5.25 mM Ca^{++} (ordinate) plotted against the ratio at 0.25 mM Ca^{++} (abscissa) measured at the same time and in the same gallbladder. Different points correspond to different gallbladders or to different times after dissection. Separate regression lines have been plotted by least squares through the data for each alkali chloride (● KCl, +RbCl, ○ NaCl, □ LiCl, △ CsCl). See text for discussion

Ca^{++} in the same gallbladder. Different values for a given salt were obtained from different gallbladders or else at different times after dissection. It is apparent that the permeability ratios for a given salt in low and in high [Ca^{++}] are linearly correlated, the correlation coefficient for the regression line being 0.99 in NaCl, 0.99 in LiCl, 0.94 in CsCl, 0.93 in RbCl, and 0.91 in KCl. The positions of the KCl and RbCl lines are clearly different

from each other and from the other lines, but the NaCl and LiCl lines are indistinguishable, and although the slope may appear to the eye slightly less for CsCl, the difference between the CsCl line and the NaCl–LiCl lines is not statistically significant ($0.1 < p < 0.25$). The lines of Fig. 10 can in fact be predicted from a model which assumes that the variation in a permeability ratio measured at different times or in different gallbladders is due to varying contributions from a free-solution shunt in parallel with undamaged epithelium (paper II, model 2). Thus, the conclusion remains that Ca^{++} affects dilution potentials in the sequence $Na^+ \sim Cs^+ \sim Li^+ > Rb^+ > K^+$.

Discussion

Since a general discussion of the ion permeation mechanism in gallbladder must be postponed until the remaining experiments have been presented in the following paper (paper IV), the present discussion will confine itself to three problems.

Concentration Dependence of Permeability Ratios and Conductance

Over a salt concentration range of at least 25 to 400 mM, the following three properties hold: conductance is a linear function of concentration (Fig. 4); there is a finite permeability to both anions and cations; and the anion/cation permeability ratio increases only slightly with concentration (Fig. 8). Even if no other experimental information were available, these results would suffice to prove that the cation permeation mechanism in the gallbladder is not the same as that in a homogeneous, macroscopic, hydratable ion-exchange membrane, whether with fixed or mobile sites [i.e., ion exchangers in the range of imperfect co-ion exclusion, discussed in detail by Teorell (1953)]. Because of the electroneutrality condition, the conductance of an ion exchanger is virtually independent of bathing-solution salt concentrations until these approach and exceed the membrane concentration of ion-exchange sites. Linear conductance-concentration curves are obtained for ion exchangers only when salt concentrations greatly exceed site concentrations, but in this range the anion/cation permeability ratio goes to the free-solution mobility ratio (*see* Fig. 1, top; Teorell, 1953, Fig. 9a). In fact, the gallbladder permeability ratios are very unlike free-solution mobility ratios and show only slight concentration dependence (the possible significance of this slight dependence is discussed in paper IV, p. 381). The findings of finite cation and anion permeabilities and a concentration-

independent permeability ratio are compatible with an ion-exchange mosaic (model 2, p. 335), but this model is ruled out by the linear conductance-concentration curve.

Implications of the Current-Voltage Relation

Since artificial thin lipid membranes break down under applied voltages of about 200 mV (Szabo, *personal communication*), it may seem initially surprising that the gallbladder should be undamaged by potentials of at least 800 mV. However, membrane strength may be increased by components such as proteins and divalent cations, which are present in biological membranes but absent from simple thin lipid membranes. For instance, algal cell membranes withstand at least 300 mV (Coster, 1965); frog choroid plexus survives at least 350 mV (Wright, *unpublished observation*); the breakdown threshold of thin lecithin membranes is raised by Ca^{++} or Fe^{++} to at least 300 mV (Läuger, *personal communication*); thin membranes of brain phospholipid extracts likely to contain protein withstand at least 600 mV (Liberman & Topaly, 1968); and thin membranes of mannolipid withstand over 750 mV (Thompson & Henn, 1970).

Thus, the high breakdown threshold of the gallbladder does not in itself argue against the possibility that permeation is controlled by a membrane thin enough to violate microscopic electroneutrality. However, this possibility is rendered unlikely by the linearity of the current-voltage relation both in symmetrical solutions and in the presence of a single-salt concentration gradient. Even in symmetrical solutions the current-voltage curve of a thin membrane containing neutral carriers is found experimentally to be nonlinear (current increases above linearity with increasing voltage: Szabo *et al.*, 1969, Fig. 1), and this effect has been interpreted theoretically in terms of image forces on an ion passing from a region of high to low dielectric constant (Neumcke & Läuger, 1969), whether or not the region of low dielectric constant contains carriers. The current-voltage relation of a thin membrane containing neutral fixed sites is found experimentally to be linear in symmetrical solutions but nonlinear in the presence of a single-salt concentration gradient (Cass *et al.*, 1970), as expected from the theoretical treatment of paper II. Eq. (105) of paper II has been used to calculate the theoretical I–V curve displayed in Fig. 11 (labeled "theory, with shunt") and predicted for a thin membrane with fixed neutral sites yielding the dilution potentials measured in the gallbladder. It is apparent from Fig. 11 that the predicted nonlinearity is so marked that it could not have escaped detection in our experiments had it existed. Given the conclusion, then,

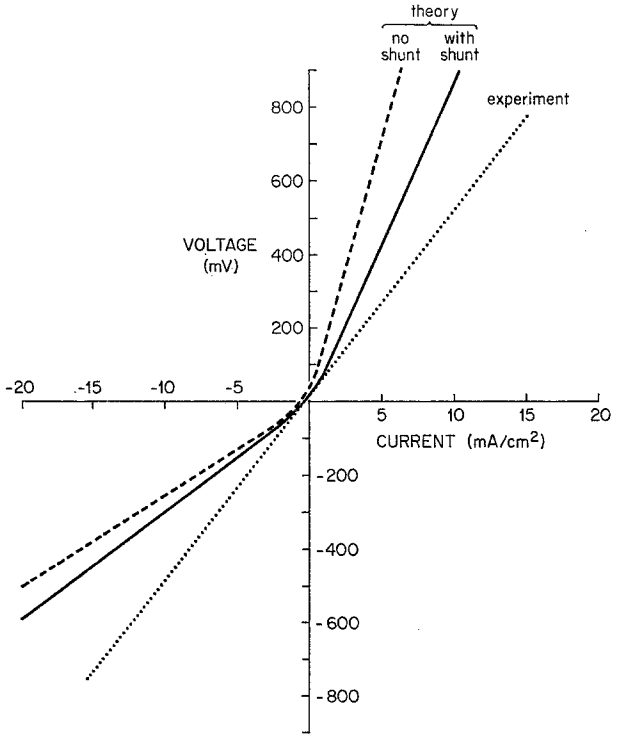

Fig. 11. Predicted current-voltage relation in asymmetrical solutions for a thin membrane with fixed neutral sites, compared with the experimental curve for the gallbladder. The curve labeled "theory, with shunt" was calculated from Eq. (105) of paper II, choosing values of the parameters to correspond to the situation of the gallbladder separating 30 and 150 mM NaCl solutions ($a'' = 115.3$ mM, $a' = 28.4$ mM, $n = 0.8$, $u_3 = 0$, $B_1 = 1.09$, and taking γ_v as the average of the two bathing-solution activity coefficients). The curve labeled "experiment" is the curve of Fig. 6b actually obtained for the gallbladder. Note that the experimental curve is linear but the theoretical curve is nonlinear, so that the membrane controlling permeation in the gallbladder cannot be thin enough for microscopic electroneutrality to be violated. The curve labeled "theory, no shunt", calculated from Eq. (100) of paper II for a thin fixed-neutral-site membrane that is perfectly cation-selective, shows that the predicted nonlinearity would be even more marked for a thin membrane without a shunt

that the permeation-controlling membrane in the gallbladder is too thick to violate microscopic electroneutrality, a linear current-voltage relation is the immediate expectation for fixed ion-exchange sites (models 1 and 2, pp. 335–336 – but these models are excluded by the considerations discussed on p. 351), for fixed neutral sites (model 4), or for neutral carriers. However, a linear current-voltage relation is not the expectation for a mobile-site ion exchanger, whose current saturates with increasing voltage (Walker & Eisen-

man, 1966), due to depletion of charged carriers at one face of the membrane (Conti & Eisenman, 1966).

Finally, the linearity of the current-voltage relation in the gallbladder makes it unlikely that the rate-limiting step to ion permeation is at a membrane-solution interface rather than in a membrane interior (*see* pp. 333–334). If the main resistance to ions were at an interfacial boundary (owing, for example, to a high activation energy for passing from water into the membrane or else to a slow chemical reaction between an ion in solution and a carrier in the membrane), the simplest prediction would be for the current to saturate with increasing voltage. (More complicated models could of course be devised in which an interfacial step coincidentally appeared to behave as an ohmic resistance, e.g., because of a potential-dependent activation energy).

Briefly, then, the current-voltage relation in the gallbladder suggests that the membrane controlling permeation is sufficiently thick for microscopic electroneutrality to have to be obeyed, and that cation permeation does not involve mobile ion-exchange sites and is not limited by an interfacial resistance.

The Ca^{++} Effect

The reduction in alkali halide dilution potentials caused by Ca^{++} is in large part due to a suppression of cation conductance (Wright & Diamond, 1968), suggesting that Ca^{++} competes with alkali cations for membrane sites. If this interpretation is correct, there will therefore be an inverse relation between the equilibrium binding constant of each alkali cation for the site and the magnitude of the reduction in the alkali halide dilution potential caused by Ca^{++}: the more strongly bound a cation, the less it will be displaced by Ca^{++}, and the less will be the reduction in the dilution potential. Hence the sequence for Ca^{++} effects on dilution potentials, $Na^+ \sim Cs^+ \sim Li^+ > Rb^+ > K^+$, implies that the equilibrium-constant sequence among the alkali cations is $K^+ > Rb^+ > Na^+ \sim Cs^+ \sim Li^+$.

In general, permeability coefficients, partial ionic conductances, and other selectivity measurements depend on two kinds of factors: (1) equilibrium affinities, such as binding constants between membrane sites or carriers and different cations; and (2) non-equilibrium factors, such as mobilities. For example, in an ion-exchange membrane the permeability ratio P_a/P_b for the ions a and b, as deduced from biionic potentials, equals the mobility ratio u_a/u_b times the binding-constant ratio K_a/K_b (Eisenman, 1965). Different physical forces enter into these two factors (*cf.* Doremus, 1967), and thus ion mobility sequences will almost always differ from equilibrium affinity

sequences in the same membrane. Where ions traverse a membrane in the unassociated form (i.e., not combined with a carrier), the mobility sequence tends to be the inverse of the equilibrium sequence: the most strongly bound ions is likely to be the least mobile. For instance, in K^+-selective glass electrodes which are 10 times more permeable to K^+ than to Na^+, K^+ is 10 times *less* mobile than Na^+ but 100 times more strongly bound, so that the permeability sequence is in the same direction as the equilibrium sequence and opposite to the mobility sequence (Eisenman, 1967).

If our interpretation of the Ca^{++} effect is correct, the sequence it yields, $K^+ > Rb^+ > Na^+ \sim Cs^+ \sim Li^+$, may be a measure of purely equilibrium affinities uninfluenced by non-equilibrium factors such as mobilities. Essentially the same sequence ($K^+ > Rb^+ > Na^+ > Cs^+ \sim Li^+$) is obtained for conductances (p. 343), which are certain to involve a non-equilibrium component in the form of a mobility. This suggests that mobility ratios among different alkali cations in the gallbladder are closer to one than are the equilibrium-constant ratios, just as in the above-cited example of the K^+-selective glass electrode. Further discussion of equilibrium selectivity and mobility selectivity in the gallbladder is postponed to paper IV.

In summary, the results presented in this paper suggest that the gallbladder is not an ion exchanger, that the controlling membrane is thick enough to be microscopically electroneutral, that interfacial effects are not rate-limiting, and that equilibrium selectivity is more important than mobility selectivity.

It is a pleasure to acknowledge our debt to: Dr. George Eisenman for many fruitful discussions; Drs. Eisenman, A. D. Grinnell, S. McLaughlin, and G. Szabo, for suggestions and comments on the manuscripts; Dr. J. Tormey, for advice on ultrastructural problems; and Mr. W. New, for design of equipment. This work was supported by U.S. Public Health Service grants GM 14772 and HE 11351 from the National Institutes of Health, and by grants from the Los Angeles County Heart Association and the Life Insurance Medical Research Fund.

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