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EFFECTS OF pH AND POLYVALENT CATIONS ON THE SELECTIVE PERMEABILITY OF GALL-BLADDER EPITHELIUM TO MONOVALENT IONS

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SUMMARY

1. From the effect of pH on KCl diffusion potentials an effective titration curve is obtained for the membrane charges controlling ion selectivity in gall-bladder epithelium. The epithelium behaves as if it is negatively charged at physiological pH (K^+ and Na^+ more permeant than Cl^-), has an isoelectric point near pH 3, and is positively charged at lower pH (Cl^- more permeant than K^+ or Na^+). This inversion of selectivity at low pH is completely reversible.

2. The positive charges responsible for selective permeability to Cl^- at low pH react with 1,5-difluoro-2,4-dinitrobenzene.

3. The relative potencies of 17 polyvalent cations in increasing P_{Cl}/P_{Na} are compared, where P stands for relative permeability coefficients of the ions indicated.

4. Low pH and high Ca^{2+} concn. both decrease cation conductance and increase anion conductance.

5. Protonation of membrane charges also affects selectivity among alkali cations: P_{Cs}/P_{Na} inverts, and P_K/P_{Na} decreases, at low pH.

6. The results are related to theories analysing inter-cation specificity in terms of the field strength of membrane charged groups.

INTRODUCTION

The gall-bladder epithelium is more permeable to most small cations than to small anions, and it also discriminates among ions of like charge. For example, permeability to K^+ is higher than to Na^+, and to Na^+ higher than to Cl^- (refs. 1–3). As with other biological membranes, the factors involved in this discrimination between ions provide an important but incompletely understood problem. The present paper explores the effects of pH and of polyvalent cations on membrane permeability to monovalent ions, taking advantage of the simple electrical behavior of the gall-bladder and of the wide range of pH and polyvalent cations that it tolerates. The stimulus to undertake this study arose from the observations that diffusion potentials and streaming potentials across the gall-bladder were influenced by Ca^{2+} concentration[3] and that streaming potentials across the small intestine were influenced by pH

Abbreviations: FFDNB, 1,5-difluoro-2,4-dinitrobenzene; p.d., potential difference.

(see ref. 4). The experimental results indicate that the effects of both these classes of agents can be simply interpreted in terms of their effect on membrane charge. Preliminary accounts of some of this work have been presented[5,6].

METHODS

The permeability of isolated gall-bladder epithelium to ions was studied electrically by measuring transepithelial diffusion potentials, streaming potentials, and conductances. Techniques used for obtaining *in vitro* preparations of rabbit gall-bladder and for measuring transepithelial electrical potential differences were similar to those described previously[1,3]. Briefly, the gall-bladder was removed from anesthetized rabbits, everted, cannulated with a polyethylene cannula, filled with a Ringer's solution, and suspended at ambient room temperature (22–26°) in a beaker of solution which was stirred vigorously with a stream of O_2 bubbles. In the everted orientation the gall-bladder consists of a sac covered on the outside with a single uninterrupted layer of columnar epithelium, supported by connective tissue and smooth muscle, which face the inside of the sac. Adjacent epithelial cells are joined at their apical borders by tight junctions, so that molecules and ions crossing the epithelium probably pass across two sets of cell membranes, one at the mucosal and the other at the serosal faces of the cells. These two sets of membranes have the same relative ionic permeability coefficients[1,3]. The solution outside the sac in contact with the apical or brush-border side of the epithelium is referred to as the mucosal solution, while that within the sac is referred to as the serosal solution. The potential difference (p.d.) across the wall of the organ was recorded by connecting the mucosal and serosal solutions to a Keithley 610B electrometer by means of salt bridges and calomel half-cells. These bridges contained either 0.15 M NaCl or satd. KCl in 4 % agar. The output of the electrometer was connected to a Varian potentiometric chart recorder, model G11A. The asymmetry potential of the circuit without the gall-bladder was checked frequently throughout the experiments. When the ionic compositions of the mucosal and serosal solutions were not identical, the junction potential was measured by means of a satd. KCl bridge. The transmural p.d.'s were then obtained by subtracting the asymmetry potential and junction potential from the measured p.d.

The conductance of the gall-bladder was measured by observing the potential drop when a direct current of 50–100 μA was passed across the tissue for approx. 30 sec. In these experiments the gall-bladder was slit open to form a flat sheet which was then clamped between two lucite chambers similar to those described by USSING AND ZERAHN[7]. The area between the chambers was 1.13 cm^2, and the volume of saline in each chamber was 10 ml. The p.d. was recorded as described above by salt bridges 1.3 mm from the gall-bladder. The current, tapped off potentiometrically from a battery and measured on a Keithley 600A electrometer, was passed through Ag–AgCl electrodes leading to opposite sides of the gall-bladder by agar–saline bridges. All conductances were corrected for the conductance of the saline between the tips of the potential-monitoring salt bridges. Experiments in which the epithelium was scraped off the gall-bladder showed that the submucosal and serosal tissue resistance was less than 6 % of the total resistance, an estimate also obtained from gall-bladders whose cell membranes had been destroyed by exposure to chloroform. Hence the conductance of the gall-bladder is largely controlled by the epithelium, as expected.

The composition of the Ringer's solution used in the majority of the experiments was 150 mM NaCl, 6 mM KCl, and 0.25 mM $CaCl_2$, buffered at pH 7.4 with 2.5 mM sodium phosphate. In some experiments mentioned specifically in the text, the phosphate buffer was replaced by 1.5 mM Tris or 0.4 mM imidazole buffers. The following buffers were used to test the effects of pH variations between 2.4 and 9.7: 1.6 mM glycine and NaOH, pH 7.7–9.7; 1.6 mM boric acid and NaOH, pH 8.3; 2.5 mM Na_2HPO_4–NaH_2PO_4 or 2.5 mM K_2HPO_4–KH_2PO_4, pH 5.0–8.0; 1.6 mM potassium phthalate and NaOH, pH 4.0–5.0; 1.6 mM potassium phthalate and HCl, pH 2.4–3.8. The ionic composition of the saline was varied by isosmotically replacing the NaCl in full or in part with KCl, choline chloride, CsCl, or sucrose. Various concentrations of divalent cations were obtained by adding aliquots of isotonic stock solutions of chloride salts of the cations (Be^{2+} as the nitrate) to the experimental saline. $LaCl_3$, $AlCl_3$, $Th(NO_3)_4$, and $UO_2(NO_3)_2$ were added to Ringer's solution to give the final desired concentration without compensating for the small shifts in osmolarity. The pH of all solutions was checked by means of a glass electrode. Solutions containing 1,5-difluoro-2,4-dinitrobenzene (FFDNB) were prepared by dissolving 100 mg FFDNB in 1 ml abs. methanol and adding drop-wise with stirring to 160 ml of Ringer's solution. Since FFDNB undergoes slow hydrolysis in water with liberation of H^+, solutions of it were made up immediately prior to being tested on a gall-bladder. The pH of the Ringer's solution containing FFDNB was measured immediately before and after exposure of the gall-bladder, and the pH drop was found to be always less than 0.4 pH unit.

All errors quoted are standard deviations.

RESULTS

Effect of pH on streaming potentials

"Streaming potential" is the term used to describe the p.d. produced by the flow of solution through a charged membrane under a hydrostatic or osmotic pressure head. Such potentials have been observed in the gall-bladder[2,3,8,9], small intestine[4], and nerve[10]. With identical solutions on both sides of the gall-bladder the transmural potential was found to be less than 1 mV serosa-positive, because this epithelium lacks electrogenic transepithelial ion pumps and the mucosal and serosal membranes have symmetrical permeability characteristics[1,3]. Under the experimental conditions (room temperature, absence of bicarbonate) the NaCl active transport mechanism of the gall-bladder was functioning at less than 18 % of the maximal rate, and is in any case an electrically neutral pump that sets up no p.d. and can be neglected for the purposes of the present study[1,2,11]. Replacing the mucosal fluid with saline identical in ionic composition to the serosal solution but containing in addition 100 mM sucrose generated a streaming potential of 3–5 mV mucosa-positive, which built up with a half-time of about 11 sec and was maintained as long as the osmotic gradient was present. The sign of the p.d. suggests that the cell membranes of the gall-bladder are negatively charged and contain an excess of positive mobile ions. The proportionality constant between these osmotic potentials and the flow rate was shown previously[12] to be about 1 mV per 2 $\mu l \cdot cm^{-2} \cdot h^{-1}$ in rabbit gall-bladder. As in artificial membranes[13], recent evidence in both gall-bladder (J. M. DIAMOND AND H. J. WEDNER, unpublished observations) and nerve[10] indicates that the measured streaming potential consists

not only of the electrokinetic flow potential itself but also contains a large boundary-diffusion-potential component, caused by flow through the membrane producing local changes in salt concentrations within the unstirred layers adjacent to the membrane: both components imply negative membrane charge.

The effect of a range of pH from 2.4 to 9.7 on the p.d.'s is illustrated in Fig. 1. A streaming potential was measured first at pH 6.85, then after the pH of the mucosal solution had been changed to some new value, and finally when the pH of the mucosal solution was changed back to 6.85. All streaming potentials in Fig. 1 are expressed as percentages of the average value of the pH 6.85 streaming potentials measured immediately before and after the test pH. For instance, the streaming potentials at pH 6.85 before and after the test pH of 4.25 were 3.85 mV and 3.90 mV, respectively, while at pH 4.25 the p.d. was 2.55 mV. Therefore the p.d. at pH 4.25 was

$$\frac{2.55 \cdot 100}{(3.85+3.90)/2} = 66\,\% \text{ of that at pH } 6.85.$$

In Fig. 1 two experiments are shown, one where the pH was varied between 6.85 and 9.7 and the second between pH 2.4 and 6.85. It can be seen that between pH 5.1 and 9.7 the p.d. was virtually independent of pH, but below pH 5.1 the p.d. decreased with decreasing pH such that at the lowest pH tested, 2.4, there was an actual reversal in the polarity (hyperosmotic solution negative instead of positive). This reversal suggests that at low pH the cell membranes became positively charged and now contained an excess of negative mobile ions. Fig. 1 also shows that the p.d. at a given pH is the same for glycine and phosphate buffers, and for phosphate and phthalate buffers.

Provided that the period of exposure to low pH was brief, the effect of pH's below 10 on gall-bladder streaming potentials was completely reversible. As judged by comparison of the preceding and subsequent streaming potential at pH's near neutrality, the effect of exposure to pH 2.4 for up to 2 min was 100 % reversible; for 5 min, about 90 % reversible; and for 12–20 min, about 60 % reversible. (This

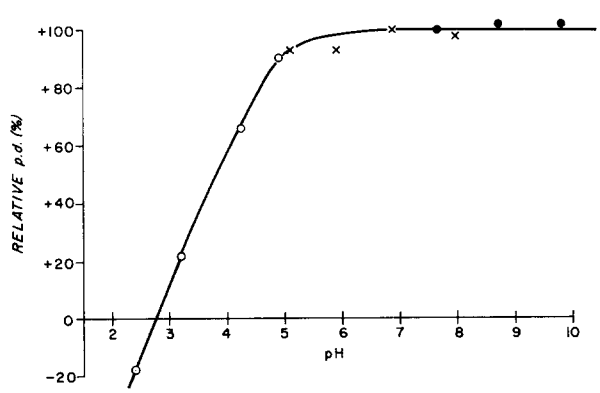

Fig. 1. Effect of pH on NaCl streaming potentials. The streaming potential resulting from addition of 100 mM sucrose to the mucosal bathing solution (NaCl Ringer's solution) was measured as a function of mucosal bathing solution pH (abscissa). The serosal solution throughout the experiment was NaCl Ringer's solution at pH 6.85. The ordinate is the streaming potential at a given pH, expressed as a percentage of the average streaming potential at pH 6.85 measured immediately before and afterwards. Note the reversal in sign of the p.d. at low pH. Buffers: ●, glycine; ×, phosphate; ○, phthalate.

reversibility is also illustrated in Fig. 2 for effects of pH on diffusion potentials.) Thus, the effect of low pH in converting the gall-bladder from a cation-permeable to an anion-permeable membrane cannot be due to irreversible and non-specific changes in the membrane. Effects of pH below 2.4 were not tested. High alkaline pH's proved more damaging: effects of exposure to pH 10.7 for 2 min were only 83 % reversible, and exposure to pH 11.6 for 2 min irreversibly abolished streaming potentials and diffusion potentials.

From Fig. 1 an isoelectric point of 2.8 can be obtained for the membrane charge responsible for ion selectivity and streaming potentials in this particular gall-bladder. These figures varied only slightly among different preparations: the average value and standard deviation for a total of five gall-bladders was 2.9 ± 0.2. Changes of pH on both mucosal and serosal sides simultaneously yielded the same streaming potential: pH curve and isoelectric point as pH changes in the mucosal solution alone (probably because H^+ is so permeant that low pH also prevails in the vicinity of the serosal cell membranes when the pH of the mucosal solution is lowered). In rat small intestine, an epithelium whose membrane properties resemble those of the gall-bladder in several respects, SMYTH AND WRIGHT[4] previously obtained a value for the isoelectric point (2.7) very close to that reported here for rabbit gall-bladder.

Effect of pH on diffusion potentials

Diffusion potentials offer the advantage over streaming potentials that they permit calculation of apparent relative permeability coefficients for ions. Diffusion potentials resulting from concentration gradients of a single salt were therefore measured as a function of pH in KCl, NaCl, and choline chloride Ringer's solutions. The results with KCl will be presented first and in more detail, since K^+ and Cl^- have similar mobilities and hydrated radii in free solution at room temperature[14].

Fig. 2 depicts an experiment in which the mucosal and serosal solutions were initially both KCl Ringer's solution at pH 7.4 and the p.d. in the absence of concentra-

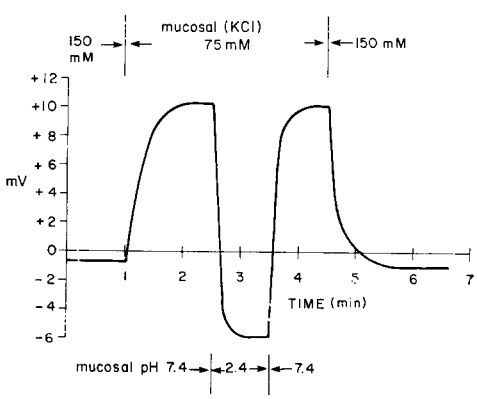

Fig. 2. Effect of pH on KCl diffusion potentials. The serosal solution throughout the experiment was KCl Ringer's solution ([KCl] = 150 mM) at pH 7.4. At the beginning and end the mucosal solution was identical in composition to the serosal solution. Between the times indicated above the record, the mucosal KCl concn. was lowered to 75 mM by isosmotic replacement with sucrose, to create a KCl concentration gradient and diffusion potential. In addition, between the times indicated below the record the mucosal pH was lowered to 2.4 while maintaining the KCl gradient. Ordinate, p.d. of the mucosa with respect to the serosa. Note that the p.d. in the absence of a gradient is near zero, and that the diffusion potential in the presence of a gradient is mucosa-positive at pH 7.4 but mucosa-negative at pH 2.4.

tion gradients was close to zero (actually, —0.8 mV, *i.e.* mucosa negative). When the mucosal solution was changed to one in which 50 % of the KCl was replaced isosmotically with sucrose, the mucosal solution went positive by 10.9 mV (from —0.7 to +10.2 mV), indicating higher permeability to K^+ than to Cl^- and suggesting that the membrane is negatively charged. The mucosal solution was next changed to one with the same lowered KCl concn. but at pH 2.4, whereupon the p.d. went negative to the initial level by 5.3 mV. This reversal in sign implies that at low pH the membrane is more permeable to Cl^- than to K^+ and has become positively charged. When the mucosal pH was changed back to 7.4, the mucosal solution again went positive by 11.0 mV, showing that the brief (60 sec) exposure to low pH had caused no damage. Finally, with full-strength KCl Ringer's solution as the mucosal solution again, the p.d. dropped back to near zero (—0.9 mV). Subsequently the small H^+ diffusion potential itself was measured by lowering the pH of the mucosal solution but not its KCl concentration, and found to be —1.0 mV, which may be subtracted from —5.3 mV to give a corrected KCl diffusion potential of —4.3 mV at pH 2.4.

The effect of a range of pH from 2.4 to 9.7 on KCl diffusion potentials, changing the pH's of both mucosal and serosal solutions in parallel so that no H^+ diffusion potentials arose, is illustrated in Fig. 3. The graph is very similar to that obtained for NaCl streaming potentials (Fig. 1), and the isoelectric point, 3.1, is close to that obtained in NaCl streaming potential experiments. When the experiment of Fig. 3 was repeated while changing the pH of only the mucosal solution, the graphs of p.d. against pH were very similar to those shown in Fig. 3. In subsequent experiments, therefore, only the pH of the mucosal solution was changed.

The average value ± S.D. of the diffusion potential for a 2:1 KCl concentration gradient was 11.4 ± 1.0 mV (10 expts.) at pH 7.4 and —4.6 ± 0.7 mV (7 expts.) at pH 2.4. Apparent values of relative permeability coefficients for gall-bladder epithelium may be calculated from the constant-field equation[15,16] in the form

$$E = \frac{RT}{F} \ln \frac{P_{Na}\gamma_{Na,m}[Na^+]_m + P_K\gamma_{K,m}[K^+]_m + P_{Cl}\gamma_{Cl,s}[Cl^-]_s}{P_{Na}\gamma_{Na,s}[Na^+]_s + P_K\gamma_{K,s}[K^+]_s + P_{Cl}\gamma_{Cl,m}[Cl^-]_m}$$

where P's are relative permeability coefficients, γ's are activity coefficients, subscripts m and s refer to the mucosal and serosal solutions, respectively, and E, R, T, and F have their usual meanings*. Application of this equation to KCl diffusion potentials yields average values ± S.D. of $P_{Cl}/P_K = 0.12 \pm 0.04$ (10 expts.) at pH 7.4 and 1.95 ± 0.23 (10 expts.) at pH 2.4. HAGIWARA *et al.*[17] similarly found P_{Cl}/P_K in barnacle muscle to invert at low pH, changing from $P_{Cl}/P_K = 0.14$ at pH 7.7 to $P_{Cl}/P_K = 6$ at pH 4.0.

Diffusion potentials in NaCl Ringer's solution also reversed sign at low pH: isosmotic replacement of half of the mucosal NaCl with sucrose made the mucosal solution 11.1 ± 0.7 mV (17 expts.) positive at pH 7.4, —4.4 ± 1.1 mV (4 expts.) negative at pH 2.4, indicating $P_{Na} > P_{Cl}$ at neutral pH but $P_{Cl} > P_{Na}$ at low pH. Diffusion potentials in choline chloride Ringer's solution were orientated so that the dilute solution was already negative at pH 7.4 (—2.9 ± 0.8 mV (6 expts.) for 50 % replacement of mucosal choline chloride with sucrose), presumably because of the choline ion being much larger than Cl^-, Na^+, or K^+, and became even more negative

* It has been shown previously that this equation appears to hold in the gall-bladder[1,3].

at pH 2.4 (-12.1 ± 1.6 mV (3 expts.)). As in the case of streaming potentials and KCl diffusion potentials, the effects of brief exposure (approx. 2 min) to pH 2.4 on NaCl and choline chloride diffusion potentials were completely reversible. Substitution

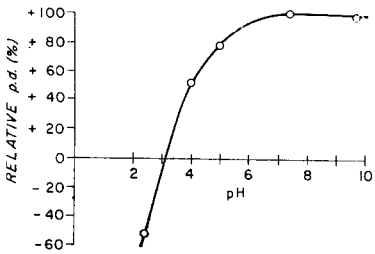

Fig. 3. Effect of different pH's on KCl diffusion potentials. Diffusion potentials were measured for a 2:1 KCl concentration gradient (serosal solution KCl Ringer's solution, mucosal solution ditto except that half the KCl had been replaced isosmotically with sucrose). At any given time the pH's of the mucosal and serosal solutions were identical to each other and were varied in parallel. The ordinate is the p.d. at a given pH, expressed as a percentage of the average diffusion potential at pH 7.4 measured immediately before and afterwards.

of the measured diffusion potentials into the constant-field equation yielded the following average values $\pm$ S.D. for apparent relative permeability coefficients: $P_{Cl}/P_{Na} = 0.12 \pm 0.03$ (17 expts.) at pH 7.4, 2.33 ± 0.87 (9 expts.) at pH 2.4; $P_{Cl}/P_{choline} = 1.53 \pm 0.21$ (6 expts.) at pH 7.4, 12.3 ± 8.3 (3 expts.) at pH 2.4. The value of P_{Cl}/P_{Na} at pH 7.4 is lower than that of 0.33 reported previously[2,3] for rabbits obtained in Boston. This proves to be a consistent difference between the rabbits obtained in Boston and in Los Angeles, since five investigators (DIAMOND, DIETSCHY, HARRISON, PIDOT, WRIGHT), using individually varying dissection techniques and white rabbits from several suppliers and working at various months of the year and in different laboratories, all obtained values close to 0.33 in Boston, while three investigators (BARRY, DIAMOND, WRIGHT) all obtained values close to 0.1 in Los Angeles. Further exploration of this difference, *e.g.* by examining several strains of rabbits, has not been undertaken.

These changes in diffusion potential with pH indicate that gall-bladder cell membranes are negatively charged and selectively permeable to cations at neutral pH but are positively charged and selectively permeable to anions at low pH.

Effect of pH on conductance

Although the diffusion potential experiments show that the ratio of anion permeability to cation permeability increases at low pH, they do not show whether an increase in absolute permeability to anions, a decrease in absolute permeability to cations, or both are involved. However, since total conductance is the sum of the partial ionic conductances and since the partial conductance of an ion is directly proportional to its absolute permeability, measurements of gall-bladder conductance provide information about the effect of pH on absolute permeabilities.

Table I summarizes conductance measurements in choline chloride Ringer's solution. From the first two rows in the table one may calculate that replacing NaCl with choline chloride at pH 7.4 lowers the conductance of the gall-bladder by 71 ± 3 % (3 expts.). This is in agreement with the decrease of 79 % predicted from relative permeabilities calculated from diffusion potentials at this pH ($P_{Cl}/P_{Na} =$

0.12, $P_{Cl}/P_{choline} = 1.53$), if G_{Cl} where G stands for the partial conductance of the ions indicated is the same in choline chloride as in NaCl. Lowering the pH of the mucosal solution from 7.4 to 2.4 in choline chloride Ringer's solution caused a large conductance *increase*, which was complete within 15 sec, stable with time, and reversible on returning to pH 7.4 (Table I). In contrast, lowering the pH of the mucosal solution (or of both the mucosal and serosal solutions) in NaCl Ringer's solution caused a *decrease* in conductance: by 37 % (3 expts.) on going from pH 7.4 to 3.4,

TABLE I

EFFECT OF pH ON GALL-BLADDER CONDUCTANCE IN CHOLINE CHLORIDE

The conductance of each of 3 gall-bladders was measured in each of 4 Ringer's solutions in the indicated sequence, beginning with NaCl Ringer's solution at pH 7.4. The mucosal and serosal solutions were changed in parallel so that their compositions were always identical to each other's. Note that conductance is lower in choline chloride than in NaCl and reversibly increases at low pH in choline chloride.

Solution	*Conductance* ($m\Omega^{-1} \cdot cm^{-2}$)		
Expt. No.	*1*	*2*	*3*
NaCl, pH 7.4	32	45	45
Choline chloride, pH 7.4	9	15	12
Choline chloride, pH 2.4	17	111	18
Choline chloride, pH 7.4	11	16	12

and by 52 % on going from pH 7.4 to 2.4. Since the ratio $P_{Cl}/P_{choline}$ increases from 1.53 to 12.3 on going from pH 7.4 to 2.4, the increase in total membrane conductance at low pH in choline chloride must involve an increase in G_{Cl}. The decrease in total membrane conductance at low pH in NaCl must result from a decrease in G_{Na} which outweighs this increase in G_{Cl}. Hence the reversal in NaCl selectivity with decreasing pH ($P_{Cl}/P_{Na} = 0.12$ at pH 7.4 and 2.33 at pH 2.4) results from a combination of a decrease in absolute cation permeability and an increase in absolute anion permeability. HAGIWARA *et al.*[17] similarly found the reversal in KCl selectivity of barnacle muscle with decreasing pH to involve both a decrease in G_K and increase in G_{Cl}.

Effect of pH on bi-ionic potentials

As in most other biological membranes, the relative permeability coefficients of the alkali cations in gall-bladder epithelium do not stand in the same ratio or even in the same sequence as the free-solution diffusion coefficients. Thus, when bi-ionic potentials were measured at pH 7.4, with NaCl Ringer's solution on the serosa and KCl, CsCl, or choline chloride Ringer's solution on the mucosa, insertion of the resultant p.d.'s (mucosa positive in the cases of CsCl and choline chloride, negative in the case of KCl) together with P_{Cl}/P_{Na} from NaCl diffusion potentials into the constant-field equation yielded apparent values of $P_K/P_{Na} = 2.23 \pm 0.49$ (8 expts.)*,

* This apparent value of P_K/P_{Na} derived from bi-ionic potentials does not agree with the ratio of the apparent values of P_K/P_{Cl} and P_{Na}/P_{Cl} derived from diffusion potentials (p. 62). On the basis of a more detailed analysis (B. H. BARRY, J. M. DIAMOND AND E. M. WRIGHT, to be published separately) this difference is interpreted in terms of the fact that a permeability coefficient possesses two components: a mobility (non-equilibrium) component, which alone determines single-salt diffusion potentials, and an equilibrium component, which together with the mobility component determines bi-ionic potentials (*cf.* EISENMAN[19]). The same considerations underlie the difference between the anion permeability sequences obtained in barnacle muscle from conductance measurements and from bi-ionic potentials by HAGIWARA *et al.*[17].

$P_{Cs}/P_{Na} = 0.58 \pm 0.03$ (4 expts.), and $P_{choline}/P_{Na} = 0.20 \pm 0.02$ (4 expts.). For comparison, the ratios of the mobilities u in free solution[14] are: $u_K/u_{Na} = 1.48$, $u_{Cs}/u_{Na} = 1.55$ ($u_{choline}$ is not known). Cesium permeability in the gall-bladder ($P_K > P_{Na} > P_{Cs}$) therefore falls out of the free-solution mobility sequence ($u_{Cs} > u_K > u_{Na}$), and K^+ and Na^+ are in the free-solution sequence but are discriminated more sharply. Since alkali cation selectivity of glass electrodes and other artificial membranes is attributable to cation interactions with membrane negative charges (EISENMAN[18]) and since membrane negative charges in the gall-bladder appear from reversal of diffusion potentials to become protonated at low pH, the effect of pH on Na^+–K^+–Cs^+ discrimination of the gall-bladder was studied.

When measured both at pH 7.4 and 2.4 in the same gall-bladder, Na–Cs bi-ionic potentials were found always to reverse in sign at low pH, such that the CsCl solution was electrically positive with respect to the NaCl solution at pH 7.4 ($P_{Na} > P_{Cs}$) but negative at pH 2.4 ($P_{Cs} > P_{Na}$). For example, in one gall-bladder in which the serosal solution was NaCl Ringer's solution and half the NaCl in the mucosal solution was changed to CsCl, the mucosal p.d. was +4.3 mV at pH 7.4 but −1.3 mV at pH 2.4, and these p.d. values combined with P_{Cl}/P_{Na} from NaCl diffusion potentials at each pH yielded $P_{Cs}/P_{Na} = 0.54$ at pH 7.4, 1.16 at pH 2.4. In a total of 5 similar experiments the average value of P_{Cs}/P_{Na} at pH 2.4 was found to be 1.17 ± 0.09 (4 expts.), as compared to 0.58 ± 0.03 (5 expts.) at pH 7.4. Thus, the Cs–Na permeability sequence becomes the same as the free-solution mobility sequence when membrane charges are protonated, but is the reverse of it at physiological pH.

K–Na bi-ionic potentials were found to remain unchanged in sign (NaCl Ringer's solution positive to KCl Ringer's solution) on going from pH 7.4 to 2.4 but to decrease in magnitude, partly due to the increase in chloride permeability and partly due to a decrease in P_K/P_{Na}. The average calculated value of P_K/P_{Na} at pH 2.4 was 1.30 ± 0.15 (6 expts.), closer to the free solution mobility ratio (1.48) than was the permeability ratio at pH 7.4 (2.23).

Effect of FFDNB

The reagents 1-fluoro-2,4-dinitrobenzene (Sanger's reagent) and 1,5-difluoro-2,4-dinitrobenzene (FFDNB) irreversibly remove positive charge from proteins and lipids by replacing an ionizable hydrogen of an amino group or of other groups with an aromatic residue. Both reagents enormously increase the permeability of human red cells to Na^+ and K^+ (but not to small water-soluble non-electrolytes), apparently by reacting with the membrane positive charges responsible for the cation impermeability of the normal erythrocyte[20]. Both reagents also affect the permeability changes to Na^+ and K^+ underlying the action potential in squid nerve and lobster nerve[21]. Since Figs. 1 and 2 suggest that the cell membranes of gall-bladder epithelium contain positive charges at low pH, the effect of FFDNB on KCl diffusion potentials in the gall-bladder was tested. The experimental procedure was to measure a diffusion potential in KCl Ringer's solution for a 2:1 KCl concentration gradient; next, to expose the gall-bladder for 15 min at either pH 7.4 or 8.3 to KCl Ringer's solution containing 2.8 mM FFDNB; and at the end of this 15-min reaction period to measure the diffusion potential for a 2:1 gradient again, first at pH 7.4 and then at pH 2.4. Average diffusion potentials for 4 gall-bladders exposed in this fashion to FFDNB were 9.0 ± 1.6 mV (8 expts.) at pH 7.4 and -2.2 ± 0.3 mV (4 expts.) at pH 2.4,

the sign being that of the dilute (mucosal) solution. In 5 control gall-bladders carried through this procedure but with FFDNB omitted, average diffusion potentials were 9.2 ± 1.1 mV (8 expts.) at pH 7.4 and −4.7 ± 0.8 mV (4 expts.) at pH 2.4. Thus, in 15 min FFDNB had no effect on the p.d.'s at pH 7.4, but reduced by 53 % the reversed diffusion potential at pH 2.4 whose existence depended upon the presence of membrane positive charges. The p.d. at pH 2.4 was lower in all 4 reacted gall-bladders (1.9–2.6 mV) than in all 5 control gall-bladders (3.4–5.3 mV). Reaction with FFDNB for longer periods of up to 1 h also reduced but did not completely abolish the reversed diffusion potentials. Control experiments with 5 mM NaF or with 0.6 % methanol in KCl Ringer's solution showed that the effect of FFDNB was not due to the liberation of F^- during the reaction or to the low concentrations of methanol present in the reaction mixture.

Effects of polyvalent cations

As reported previously[3], the magnitude of NaCl diffusion potentials and streaming potentials across the gall-bladder at pH 7.4 decreases with increasing Ca^{2+} concentration. This observation has now been extended to 16 other polyvalent cations, and its basis has been explored by means of conductance measurements.

Fig. 4 shows that when the mucosal Ca^{2+} concn. is increased from 0.25 to 5.25 mM, the diffusion potential resulting from a 2:1 NaCl concentration gradient at pH 7.4 is reversibly reduced by several mV. The change in p.d. represents a change in the NaCl diffusion potential due to an increase in P_{Cl}/P_{Na}, rather than a diffusion potential due to the small concentration gradient of calcium itself, since NaCl diffusion potentials were also reduced when the Ca^{2+} concn. was changed symmetrically on both sides of the gall-bladder (*cf.* DIAMOND AND HARRISON[3]). From the change in

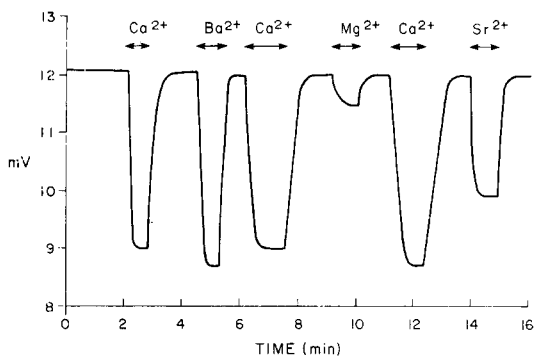

Fig. 4. Effects of the four alkaline earth cations upon NaCl diffusion potentials. A diffusion potential of about 12 mV resulting from a 2:1 NaCl concentration gradient was established (serosal solution NaCl Ringer's solution, pH 7.4, $[Ca^{2+}] = 0.25$ mM; mucosal solution ditto except that half of the NaCl had been replaced isosmotically with sucrose). At the indicated times the effect of the indicated alkaline earth cation at a mucosal concentration of 5 mM (in addition to the 0.25 mM Ca^{2+} which was always present) was tested on this diffusion potential, by addition of 4.75 ml of an isotonic (105.3 mM) solution of X^{2+} Cl_2 to 95.25 ml of mucosal solution. Note that the diffusion potential is depressed markedly by Ba^{2+} and Ca^{2+}, less by Sr^{2+}, and least by Mg^{2+}. (The figure is a trace of the measured voltage before correction for junction potentials. Junction potential correction lowers the level of the whole trace by 2.4 mV, and raises the level during application of an alkaline earth by 0.7 mV with respect to the rest of the trace, so that Mg^{2+} proves to have practically no effect at this correction.)

p.d. (an average decrease of 28 % in 7 gall-bladders), one may calculate that this change in Ca^{2+} concn. increases the apparent value of P_{Cl}/P_{Na} on the average from 0.12 to 0.28. At pH 2.4, where the sign of NaCl diffusion potentials is reversed (dilute solution negative, $P_{Cl}/P_{Na} > 1$), increased Ca^{2+} concn. increased the magnitude of these reversed diffusion potentials, again implying an increase in P_{Cl}/P_{Na}. The effect of Ca^{2+} on choline chloride diffusion potentials at pH 7.4 was analogous to that on NaCl diffusion potentials at pH 2.4, since in this case too the cation is more permeant than the anion. In 5 experiments, increasing mucosal Ca^{2+} concn. from 0.25 to 5.25 mM increased the p.d. resulting from a 2:1 choline chloride gradient at pH 7.4 from 3.5 $\pm$ 0.6 mV to 5.3 $\pm$ 0.9 mV (dilute solution negative). The corresponding calculated increase in $P_{Cl}/P_{choline}$ is from 1.60 $\pm$ 0.14 to 2.21 $\pm$ 0.30.

To decide whether the calcium effect involves a decrease in P_{Na}, increase in P_{Cl}, or both, membrane conductance was measured in 3 gall-bladders in NaCl Ringer's solution (substituting Tris for phosphate buffer) when the mucosal Ca^{2+} concn. was raised from 0.25 to 5.25 mM. In all 3 experiments there was a conductance decrease which occurred within 7 sec, remained stable as long as the Ca^{2+} concn. was maintained at 5.25 mM, and was reversible on returning to 0.25 mM. Values for this conductance decrease in three gall-bladders were from 48.8 ($[Ca^{2+}] = 0.25$ mM) to 35.7 ($[Ca^{2+}] = 5.25$ mM), 36.4 to 27.4, and 37.0 to 23.8 $m\Omega^{-1} \cdot cm^{-2}$. This decrease in conductance (by 29 %, on the average) caused by raised Ca^{2+} concn. is similar to that caused by low pH and must be due to a decrease in sodium absolute permeability. Comparison of the magnitude of this conductance decrease with the magnitude of the change in P_{Cl}/P_{Na} indicated that there might also be a small increase in G_{Cl} masked by the larger decrease in G_{Na}. That Ca^{2+} does increase G_{Cl} was shown more clearly by the fact that in choline chloride Ringer's solution, where anion conductance is relatively more important than in NaCl, increasing Ca^{2+} concn. from 0.25 to 5.25 mM in 5 experiments increased membrane conductance in all cases (on the average, by 13 $\pm$ 6 %).

In other epithelia complete removal of Ca^{2+} by prolonged treatment with EDTA has been shown to cause adjacent epithelial cells to separate[22–24]. Although decreasing Ca^{2+} concn. from 5.25 to 0.25 mM reversibly *decreases* P_{Cl}/P_{Na} in the gall-bladder and thus improves ion discrimination and increases p.d.'s, decreasing Ca^{2+} concn. further by brief exposure to EDTA reversibly *increases* P_{Cl}/P_{Na}, and prolonged exposure to calcium-free solutions irreversibly destroys selective permeability altogether, as judged from p.d.'s and greatly increased tracer fluxes of normally impermeant nonelectrolytes. The effects of changes in Ca^{2+} concn. between 5.25 and 0.25 mM and between 0.25 and 0 mM are therefore qualitatively different. Probably the former concentration range, the one used in the present paper, affects only membrane properties, and cell separation occurs only at lower concentrations of Ca^{2+}.

Effects of the following 17 polyvalent cations in increasing P_{Cl}/P_{Na} were compared: from group 2A of the periodic table, Be^{2+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}; from group 3A, Al^{3+}; the transition metals Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}; Zn^{2+}, Cd^{2+}, and Hg^{2+}; the lanthanide La^{3+}; and the actinides $(UO_2)^{2+}$ and Th^{4+}. The procedure was to compare the effects of various cations, at a mucosal concentration of 2.5 or 5 mM, on the diffusion potential resulting from a 2:1 NaCl gradient or on the streaming potential resulting from a 100 mM sucrose gradient, testing Ca^{2+} before and after each other cation. Irreversible effects on gall-bladder potential differences

after brief (<45 sec) exposures to polyvalent cations were noted only for Hg^{2+} and rarely for Cu^{2+}. This experimental protocol is illustrated in Fig. 4, in which the 4 alkaline earths were being compared. It is apparent that Ba^{2+} is slightly more potent than Ca^{2+}, Sr^{2+} less potent, and Mg^{2+} least in that particular gall-bladder.

The results obtained on 8 gall-bladders for effects on diffusion potentials are summarized in Table II, which gives the p.d. with 2.5 mM (or 5 mM) of the given ion added as a percentage of the p.d. with only 0.25 mM Ca^{2+} present. While there are minor variations in sequence of effects between different gall-bladders, the general pattern for the apparent sequence of decreasing effect on the p.d. is $Th^{4+} > (UO_2)^{2+} > Al^{3+} > La^{3+}$, $Cu^{2+} > Ba^{2+}$, $Ca^{2+} > Mn^{2+}$, $Be^{2+} > Sr^{2+} > Cd^{2+}$, Zn^{2+}, Fe^{2+}, $Co^{2+} > Mg^{2+}$, Hg^{2+}, Ni^{2+}. While the percentage reduction was somewhat greater in diffusion potentials than in streaming potentials, sequences of effects of various ions on both kinds of potential differences were closely similar, particularly in 3 experiments where effects on streaming potentials and on diffusion potentials were determined in the same gall-bladder.

Interpretation of some of these results requires consideration of pH changes and ion reactions in aqueous solution.

TABLE II

EFFECTS OF POLYVALENT CATIONS UPON NaCl DIFFUSION POTENTIALS

Effects of polyvalent cations were tested in 8 gall-bladders on the diffusion potential resulting from a 2 : 1 NaCl concentration gradient (serosal solution NaCl Ringer's solution, pH 7.25, imidazole or Tris buffer, $[Ca^{2+}] = 0.25$ mM; mucosal solution ditto except that half of the NaCl had been replaced isosmotically by sucrose). The table gives, for each gall-bladder, the p.d. with a cation added to the mucosal solution (as the chloride or nitrate salt) at 5 mM (gall-bladders 1–6) or at 2.5 mM (gall-bladders 7–8) as a percentage of the average p.d. measured immediately before and afterwards without added cations. This procedure is illustrated in Fig. 4. A relative p.d. of 100 means that the cation had no effect (*e.g.*, Mg^{2+}, Hg^{2+}, Ni^{2+}); a value of 60 means that the p.d. was reduced by 40%; and the negative values with Th^{4+} mean that the sign of the p.d. was actually reversed. The second column gives the pH of the mucosal solution after addition of the cation, which in some cases significantly altered the pH.

Cation added	*pH*	*Relative p.d.*							
	Expt. No.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Be^{2+}	4.45	69	77						
Mg^{2+}	7.3	101	97	100					
Ca^{2+}	7.3	63	68	77	75	68	76	82	85
Sr^{2+}	7.3	79	72	86	87	82	83		
Ba^{2+}	7.3	69	71	74	79	69	73		
Mn^{2+}	7.3	74	74	78	75	70	74		
Fe^{2+}	5.2	90	91	77					
Co^{2+}	7.15	96	82	96					
Ni^{2+}	6.8	99	96	102					
Cu^{2+}	4.95	35	39	—					
Zn^{2+}	6.8	82	85	94					
Hg^{2+}	6.7	101	96	102					
Cd^{2+}	7.15	83	83	92					
La^{3+}	7.2							57	72
Al^{3+}	4.3							25	49
$(UO_2)^{2+}$	3.2							2	14
Th^{4+}	3.0							−28	−58

The pH's of the solutions containing 9 of the ions were below 6.8 because these ions undergo reactions liberating H^+ in aqueous solution and the ion concentrations were higher than the buffer concentrations (0.4–2.5 mM) used (in many of these cases the ions would have been insoluble at pH 7 and can be dissolved as cations only at lower pH's, so that titration back to pH 7 would have been fruitless). In the cases of Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Hg^{2+} the pH's were in a range (4.95–6.8) where pH *per se* has little effect on the p.d.'s of gall-bladder (*cf.* Figs. 1 and 2), so that the effects of these ions on the p.d.'s must still be due to the ions and their transformation products. In the cases of Al^{3+}, $(UO_2)^{2+}$, Th^{4+}, and Be^{2+} the pH values *per se* (<4.5) would be expected to reduce the p.d.'s, making it difficult to draw firm conclusions about direct effects of these 4 ions independent of pH. To varying extents in all 4 cases, however, the reduction in the p.d. was greater than would be expected from the effect of pH alone, implying that the transformation products of the ions were also exerting a direct effect.

All of the deviant pH's noted in the table are indicative of oxidation, hydrolysis, and/or complex formation of these ions in aqueous solution[25–27]. Results obtained with these 9 deviant cations must in general be treated with caution in interpreting their effects on biological phenomena. For example, the low pH of solutions of uranyl salts is due to hydrolysis by the reactions

$$2(UO_2)^{2+} + H_2O \leftrightarrows (U_2O_5)^{2+} + 2H^+$$
$$(UO_2)^{2+} + (U_2O_5)^{2+} + H_2O \leftrightarrows (U_3O_8)^{2+} + 2H^+$$

Th^{4+}, Be^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, Cu^{2+}, Hg^{2+}, and probably Fe^{2+} also undergo hydrolysis to yield a variety of transformation products. The most stable form of copper in solutions of $CuCl_2$ is actually the anion $(CuCl_4)^{2-}$, whose interactions with membrane positive charges may explain the apparent effect[28] of low "Cu^{2+}" concentrations on chloride permeability in frog skin. Fe^{2+} undergoes oxidation by the reaction $Fe^{2+} + 2H_2O + {}^1/_2O_2 \rightarrow Fe(III)(OH)_3 + H^+$.

In summary, the large effect of Cu^{2+} and the small effects of Zn^{2+} and Fe^{2+} on P_{Cl}/P_{Na} are due to these ions themselves and their transformation products rather than to the small associated shifts of pH, but are not necessarily due to the native (divalent) form of the ion. Hg^{2+} and Ni^{2+}, which may undergo limited hydrolysis, have little or no effect on P_{Cl}/P_{Na}. The large effects of Al^{3+}, $(UO_2)^{2+}$, Th^{4+}, and Be^{2+} are due partly to shifts in pH, probably partly as well to transformation products of the ions, and probably not at all to the native ions themselves. The remaining 8 ions do not undergo transformation or cause pH shifts in aqueous solution and are responsible in the native form for depressing NaCl diffusion potentials (increasing P_{Cl}/P_{Na}) with the relative potency $La^{3+} > Ba^{2+}$, $Ca^{2+} > Mn^{2+} > Sr^{2+} > Cd^{2+}$, $Co^{2+} > Mg^{2+}$.

DISCUSSION

Cation-vs.-anion selectivity

The obvious similarity between Fig. 3 (effect of pH on KCl diffusion potentials) and the titration curve of an amphoteric substance suggests that the cell membranes of gall-bladder epithelium contain both acidic and basic groups; that the effective isoelectric point is about 3.0; that the pK_a's of both the acidic and the basic groups

are in this same range (approx. pH 2 to pH 4), since more divergent pK_a's would have made themselves apparent as a two-stage titration curve, and since the absence of an FFDNB effect on p.d.'s measured at pH 7.4 points to the basic groups being in the neutral form at this pH; and that these membrane charges are responsible for the observed differences in permeability between cations and anions. That the effect of low pH on permeability is in fact due to protonation of membrane charges rather than to protein denaturation or other side-effects is clear from the complete reversibility of the permeability changes in the gall-bladder after brief exposures to low pH, and from the fact that the gall-bladder at low pH behaves as an anion-selective membrane ($P_{Cl} > P_K$) rather than as a dead membrane ($P_{Cl} \simeq P_K$). We may write the ionization of the acidic site in the form $AH \leftrightarrows A^- + H^+$, and, for the basic site, $BH^+ \leftrightarrows B + H^+$. At pH's near neutrality the sites would be in the form A^- and B, so that the membrane would bear a net negative charge, be relatively impermeable to anions, and function as an ion-exchange membrane for cations. At acidic pH's both sites would become protonated to the forms AH and BH^+, so that the membrane would bear a net positive charge, be relatively impermeable to cations, and function as an ion-exchange membrane for anions. Qualitatively similar effective titration curves for membrane charges controlling ion permeability have been obtained in frog skin from KCl diffusion potentials[29] (isoelectric point approx. 5.1), in rat small intestine from streaming potentials[4] (isoelectric point approx. 2.7), and in several endothelial membranes from electroosmotic flows[30] (isoelectric points about 4.3–5.3). In all these cases the empirical observation is that the membrane changes from cation-selective at high pH to anion-selective at low pH. These charged groups controlling permeation probably lie within the substance of the membrane itself, rather than on its external surface (*i.e.*, their counterions lie within rather than outside the surface of shear), as is made clear by the example of the erythrocyte, whose surface and internal membrane charges are of opposite signs: the surface of the erythrocyte is negatively charged at neutral pH from electrophoretic evidence (probably due to sialic acid[31,32]), but the erythrocyte is nevertheless far more permeable to anions than to cations, implying that positive charges (possibly ε-amino groups of lysine[20]) whose counterions lie within the surface of shear govern permeation.

While the reversible inversion in the value of the ratio P_{Cl}/P_K or P_{Cl}/P_{Na} at low pH is the most direct evidence for this model of an amphoteric ion-exchange membrane, the conductance measurements and the effects of polyvalent cations are also consistent with it. Lowering the pH simultaneously increases the partial conductance of chloride (G_{Cl}) by creating positively charged anion-exchange sites, and decreases G_{Na} by eliminating negatively charged cation-exchange sites. The effects of pH on barnacle muscle[17] are qualitatively very similar in that P_{Cl}/P_K inverts around pH 4.5 (a higher isoelectric point than in the gall-bladder) with both an increase in G_{Cl} and decrease in G_K. An increase in G_{Cl} and decrease in G_K with decreasing pH is also seen in crayfish muscle[33]. By blocking negatively charged sites, calcium and other polyvalent cations reduce G_{Na} (hence increasing P_{Cl}/P_{Na}). Calcium in addition increases G_{Cl} as a result of the adsorbed calcium itself acting as an anion-exchange site, as shown by the increase in conductance when the Ca^{2+} concn. is raised in choline chloride solutions. As in lobster nerve[24], H^+ and calcium probably block the same acidic sites, since the effects on permeability and conductance of lowered pH and raised Ca^{2+} concn are qualitatively the same. Ca^{2+}, Sr^{2+}, and Ba^{2+} have

similarly been shown to reduce KCl diffusion potentials in tooth enamel, which is normally cation-selective, and high concentrations of these divalent cations actually reverse the potential differences and make enamel anion-selective[35], as does low pH in the previously cited tissues.

The chemical identity of the acidic and basic groups in gall-bladder membranes cannot yet be determined unequivocally. The low pK_a of the acidic site (probably in the pH range 2–4) is compatible with either a carboxyl or a phosphoric acid residue. The basic site, which also appears to have a pK_a in this same range, can hardly be other than a nitrogen function. This low pK_a of the basic site is correlated with its relatively low reactivity to FFDNB, which reduced by half but did not abolish the reversed diffusion potentials after reaction for 15 min at pH 7.4 or 8.3. The effect of FFDNB on ion permeability is more profound in erythrocytes, which are anion-permeable and cation-impermeable at neutral pH due to basic groups with a pK_a presumably well above pH 7. The reason for this correlation between the pK_a and the reactivity to FFDNB of a basic site is that both are influenced by the availability of electrons on the site: the more readily available electrons are to protons (hence a high pK_a), the more available they may be to FFDNB for reaction. The failure of FFDNB to affect the potential differences measured at neutral pH argues against the presence of amino functions with a pK_a on the alkaline side of neutrality (*i.e.*, that would contribute positive charge at neutral pH). A speculative possibility is that sialic acid, which has a carboxyl and a substituted amino group with pK_a's both in the range 2–3 (ref. 36), provides both the acidic and basic sites in the gall-bladder. The pH-dependence of the electrophoretic mobility of the erythrocyte, whose surface charge arises from sialic acid[31,32], is very similar to the 'pH-titration curve' for the charged groups discriminating K^+ and Cl^- in the gall-bladder (Fig. 2).

Sodium–potassium selectivity

Much evidence has accumulated from studies on artificial membranes that membrane charge controls discrimination among ions of like charge as well as controlling discrimination between cations and anions. The most detailed evidence has come from studies on glass electrodes for the alkali cations. Although the 5 alkali cations (Li^+, Na^+, K^+, Rb^+, Cs^+) can be permuted to yield 5 factorial=120 different sequences, only 11 of these permutations are actually observed as selectivity sequences in artificial membranes, despite the wide range of membrane materials and glass composition tested. EISENMAN[18] observed that the selectivity sequence of the glass electrodes varied in a systematic manner with glass composition in such a way as to indicate that cation selectivity was controlled by the field strength of the negative fixed charge sites in the glass. For negative sites with very high field strengths the ion–site interaction was much stronger than the ion–water interaction, so that the relative affinities of the ions were in the order of their non-hydrated radii; while the affinities were in the opposite sequence (the order of the hydrated radii) for sites with very weak field strengths, since the ion–site interaction was then much weaker than the ion–water interaction. From a simplified model in which field strength (or the radius of an assumed monopolar anion equal in field strength to that of the actual multipolar site) was taken as the controlling variable and the Coulomb energies of interaction of the alkali cation with the site were compared with the cations' free energies of hydration, EISENMAN[39] was able to predict correctly the 9 cation se-

quences, intermediate between those of the hydrated and non-hydrated radii, actually observed in artificial membranes including the glass electrodes. The fact that the permeability sequences for alkali cations observed to date in biological membranes belong to these same 11 permutations implies that the same formulation is applicable to biological membranes[19].

The experimental observations on the pH dependence of the relative K^+, Na^+, and Cs^+ permeabilities in the gall-bladder are in qualitative accord with this interpretation that cation selectivity sequences or ratios differing from those of free-solution mobilities depend upon interactions with membrane negative charges. As the pH is lowered and membrane negative charges become protonated, the ratio P_{Cs}/P_{Na} is observed to invert: at neutral pH Na^+ is more permeant than Cs^+, whereas at low pH Cs^+ is more permeant. P_K is greater than P_{Na} at neutral as well as at low pH, but the numerical value of the ratio P_K/P_{Na} decreases with decreasing pH. The pattern of these changes is that the permeability ratios move closer to the ratios of the free-solution mobilities as the membrane negative charges are gradually protonated and their field strength is decreased. A model for the cation permeability sequence and its pH dependence in the gall-bladder can be found in the glass electrode NAS 28.3–9.7 (ref. 18, Fig. 3), in which the sequence is also $P_K > P_{Na} > P_{Cs}$ at pH 7.4 but $P_K > P_{Cs} > P_{Na}$ at pH 2.4.

Divalent cation specificity

The nominal sequence of potency of polyvalent cations in increasing P_{Cl}/P_{Na} in the gall-bladder may be compared with nominal sequences of polyvalent cation specificity in some other biological phenomena. In barnacle muscle HAGIWARA AND TAKAHASHI[40] obtained the following sequence for relative potency in mimicking the ability of Ca^{2+} to maintain the rate of rise of the action potential: La^{3+}, $(UO_2)^{2+} > Zn^{2+}$, Co^{2+}, $Fe^{2+} > Mn^{2+}$, $Ni^{2+} > Ca^{2+} > Mg^{2+} > Sr^{2+}$. The sequence Co^{2+}, $Ni^{2+} > Ca^{2+}$, $Mn^{2+} > Mg^{2+}$, Sr^{2+} has been obtained for relative potency in maintaining the resting potential of frog muscle[41]. These two muscle sequences differ from the gall-bladder sequence most notably in the relative positions of the transition metals and the alkaline earths (transition metals generally more potent than alkaline earths in muscle, less potent in gall-bladder).

A simplified theory of specificity for the four alkaline earths (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) in a cation-exchange membrane with univalent negatively charged sites has been worked out by SHERRY[42] along the lines of EISENMAN's treatment for the alkali cations. Specificity is considered to depend upon the values of two parameters: the field strength of the anionic site (or the radius of an assumed monopolar anion whose field strength would be equal to that of the actual multipolar site), and the distance between adjacent sites. Specificity is determined, as in EISENMAN's model, by the difference between the free energy of hydration of the alkaline earth cations and their Coulomb energies of interaction with the negatively charged sites, very weak sites yielding specificities in the order of the hydrated radii of the cations at any site spacing, and very strong sites yielding the sequence of the non-hydrated radii until large site spacings are reached. SHERRY's model predicts that out of the 24 sequences obtainable by permutation of the four alkaline earths, only seven should actually be observed as selectivity sequences. The five selectivity sequences listed by BUNGENBERG DE JONG[43] for effects of the alkaline earths on the electrophoretic velocity of carboxyl, phosphate,

and sulfate colloids ($Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$; $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$; $Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+}$; $Ca^{2+} > Mg^{2+} > Ba^{2+} > Sr^{2+}$; $Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$) all belong to these seven predicted patterns. In experiments on effects of the alkaline earths on P_{Cl}/P_{Na} in twelve gall-bladders, we observed two different sequences: $Ba^{2+} > Ca^{2+} > Sr^{2+} > Mg^{2+}$ in three gall-bladders, and $Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+}$ in nine gall-bladders. As in BUNGENBERG DE JONG's experiments on colloids, these sequences in the gall-bladder reflect equilibrium selectivity unaffected by mobility selectivity. Both gall-bladder sequences are among the five observed by BUNGENBERG DE JONG and the seven predicted by SHERRY and are series II and series III, respectively, in SHERRY's terminology. In terms of SHERRY's model these sequences imply that the negatively charged sites in the gall-bladder are relatively close together (less than about 5 Å apart) and have moderate field strengths, and that sites in preparations exhibiting series III have slightly closer spacing or higher field strengths than those exhibiting series II.

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