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An Estimate of the Salt Concentration in the Lateral Intercellular Spaces of Rabbit Gall-Bladder during Maximal Fluid Transport

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Summary. The ability of the gall-bladder to transport water between identical bathing solutions depends on active NaCl transport, which is thought to maintain the salt concentration in the lateral intercellular spaces above bathing solution levels and thus to create a local osmotic gradient. The mean value of this gradient has been estimated by an electrical procedure, based on measuring the small diffusion potential resulting from this gradient and from the preferential cation permeability of the gall-bladder. The electrical potential difference (p.d.) in maximally transporting rabbit gall-bladders is 1.4 mV, mucosal-solution positive to serosal solution. This p.d. is reversibly abolished or greatly reduced by six procedures which abolish or greatly reduce fluid transport (low temperature, replacement of Cl^- by SO_4^{--}, replacement of Cl^- and HCO_3^- by SO_4^{--}, replacement of Na^+ by choline, removal of HCO_3^-, and metabolic poisoning). The p.d. is increased by symmetrical partial replacement of NaCl by sucrose, which is expected to increase the salt concentration gradient between the lateral spaces and the bathing solutions. Since the transport mechanism of the gall-bladder is a neutral NaCl pump that cannot produce a p.d. directly, it is concluded that the observed p.d. is the expected diffusion potential. From this diffusion potential and from the measured value of a diffusion potential resulting from a known NaCl concentration gradient, the mean concentration of NaCl in the lateral spaces is calculated to be of the order of 10 mM above the bathing solution value. Comparison of the external osmotic gradient required to stop water flow with the p.d. recorded under this condition of zero flow supports the validity of interpreting the p.d. in this fashion as a measure of the excess local salt concentration.

One of the basic properties of epithelia is the ability to generate net fluxes of water and of specific solutes between identical bathing solutions. In many cases, the ratio of the water flux to the solute flux is such that the transported fluid has the same osmolarity as the bathing solutions. This process is termed isotonic fluid transport and is exemplified by the absorption of the digesta in the duodenum, the reabsorption of the glomerular filtrate in the renal proximal tubule, the secretion of bile by the liver, and the reabsorption of bile by the gall-bladder.

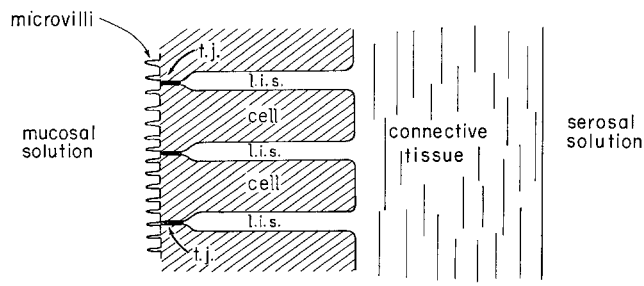

Fig. 1. Schematic diagram of gall-bladder epithelium (not to scale). Adjacent epithelial cells are separated by long, narrow, lateral intercellular spaces, *l.i.s.*, open at the end facing the serosal solution but sealed by tight junctions, *t.j.*, at the end facing the mucosal solution. Salt and water are transported from the mucosal solution to the serosal solution, apparently first passing from the mucosal solution into the cells, then from the cells into the lateral spaces

During the past decade it has been shown that water transport by epithelia is a passive consequence of active solute transport (Curran & Solomon, 1957; Windhager, Whittembury, Oken, Schatzmann, & Solomon, 1959; Diamond, 1962*c*, 1964*a*, 1965, 1968). "Black-box" physiological experiments have shown that the coupling between water and solute depends on local osmotic forces. Actively transported solute is dumped into confined regions within the epithelium and adjacent to the cell membranes, making the regions locally hypertonic, and water crosses the cell membranes as a result of these local osmotic gradients established by active solute transport (Ogilvie, McIntosh, & Curran, 1963; Diamond, 1964*b*, 1965). In rabbit gall-bladder, correlated physiological and anatomical experiments have made it possible to identify these sites of local osmosis with the lateral intercellular spaces between adjacent epithelial cells (Fig. 1; Diamond & Tormey, 1966; Kaye, Wheeler, Whitlock, & Lane, 1966; Tormey & Diamond, 1967). The geometry of these spaces makes it likely that they function as standing-gradient flow systems, in which the osmolarity decreases from a maximally hypertonic value near the tight junction towards isotonicity at the open (serosal) ends of the spaces (Diamond & Bossert, 1967, 1968).

The problem remains of determining the actual magnitude of the local osmotic gradients within epithelia. The complete solution to this problem requires the development of microanatomical methods for quantitating the concentrations of diffusible solutes while preventing their translocation. Since this direct approach is technically difficult, it seemed desirable to obtain first an estimate of the order of magnitude of the expected gradients by some less direct means. The purpose of this paper is to obtain such an

Table 1. *Composition of experimental*

Solution	NaCl	Choline Cl	$NaHCO_3$	Choline HCO_3	Na_2SO_4	KCl	$CaCl_2$	$MgSO_4$
A	132.5	–	–	–	–	7.0	1.0	1.2
B	–	–	–	–	–	7.0	1.0	1.2
C	110.0	–	25.0	–	–	7.0	1.0	1.2
D	60.0	–	25.0	–	–	7.0	1.0	1.2
E	–	–	–	–	109	–	–	1.2
F	–	110.0	–	25.0	–	7.0	1.0	1.2
G	–	–	25.0	–	94.0	–	–	1.2
H	110.0	–	25.0	–	–	7.0	1.0	1.2

estimate for rabbit gall-bladder, in which water transport is coupled to active NaCl transport.

The principle underlying this study is that the gall-bladder is more permeable to cations than to anions, so that salt concentration gradients give rise to diffusion potentials in which the dilute solution goes electrically positive with respect to the concentrated solution (Diamond, 1962*b*; Diamond & Harrison, 1966; Wright & Diamond, 1968). Active NaCl transport is thought to establish an NaCl concentration in the lateral spaces of the gall-bladder which is higher than that in the mucosal bathing solution, and should therefore create a local NaCl concentration gradient across the epithelium even when the external bathing solutions are identical. The active transport mechanism itself is a neutral NaCl pump in the gall-bladder and gives rise directly to no electrical potential difference (p.d.), unlike the situation in most other epithelia (for detailed discussion of the evidence, *see* Diamond, 1962*b*, 1968; Wheeler, 1963; Dietschy, 1964). Thus, in a maximally transporting gall-bladder separating identical bathing solutions, one should observe a mucosa-positive p.d. which is a diffusion potential due to the raised salt concentration in the lateral spaces; this p.d. should be absent in nontransporting gall-bladders; and the magnitude of the p.d. should yield an estimate of the salt concentration in the lateral spaces. Previous studies on the gall-bladder made it clear that such a p.d., if it existed at all, would be very small, but there was at least one positive indication for its existence; Whitlock and Wheeler (1964) observed in maximally transporting rabbit gall-bladders a p.d. of about 1.7 mV that appeared to have some properties of the expected diffusion potential. Hence, the experiments reported here consist of careful comparisons of transepithelial p.d. in transporting and nontransporting gall-bladders with symmetrical bathing solutions, using each gall-bladder as its own control. In addition, measurements of the external osmotic gradient required to

solutions (in mmoles/kg water)

Glucose	NaH_2PO_4	Na_2HPO_4	KH_2PO_4	K_2HPO_4	$CaSO_4$	Mannitol	Sucrose
11.1	0.375	2.125	—	—	—	—	—
11.1	0.375	2.125	—	—	—	248.3	—
11.1	1.2	—	—	—	—	—	—
11.1	1.2	—	—	—	—	—	91.1
11.1	—	—	0.375	2.125	8.0	—	—
11.1	—	—	1.2	—	—	—	—
11.1	—	—	0.375	2.125	8.0	—	—
11.1	1.2	—	—	—	—	—	200

stop fluid transport were obtained, since the p.d. and the imposed gradient during this state of zero flow yield independent estimates of the mean salt concentration in the lateral spaces. Comparison of these two estimates thus provides a test of the validity of calculating local salt concentration in this indirect fashion from p.d.

Methods

Techniques used for obtaining in vitro preparations of rabbit gall-bladder and for measuring transepithelial p.d. were in general similar to those described previously (Diamond, 1962*b*; Diamond & Harrison, 1966; Wright & Diamond, 1968). Briefly, gall-bladders were removed from anesthetized male white rabbits; these were everted, cannulated with a polyethylene cannula, filled with a Ringer's solution, and suspended at ambient room temperature (23 ± 1 °C) in a beaker of Ringer's solution stirred with oxygen bubbles (or with 95 % $O_2 - 5$ % CO_2 if the bathing solution contained bicarbonate) saturated with water vapor. Transepithelial p.d. were measured to ± 0.05 mV by connecting the mucosal and serosal bathing solutions to a Keithley 610 B electrometer and Varian G11A potentiometric chart recorder via calomel half-cells and salt bridges containing 0.15 M NaCl (or 0.11 M Na_2SO_4 for experiments using Na_2SO_4 Ringer's solutions) in 4 % agar. The mucosal bathing solution is in contact with the free surface of the epithelium (facing the gall-bladder lumen in the natural orientation); the serosal bathing solution is in contact with the connective tissue layer (facing the outside in the natural orientation). Fluid transport is in the mucosal-to-serosal direction (*see* Fig. 1). In this paper, the p.d. is always expressed as that of the mucosal solution with respect to the serosal solution.

The asymmetry potential of the circuit without the gall-bladder (generally less than 0.50 mV) was frequently checked throughout each experiment and subtracted from the measured p.d. At the beginning and end of each experiment, the diffusion potential at room temperature resulting from a 2:1 concentration gradient of NaCl was measured, using solution A as the serosal solution and a 1:1 mixture of solutions A and B as the mucosal solution (*see* Table 1). Since all concentrations cited are molal and since the contribution of mannitol to the solution volume was not negligible, the 1:1 mixture was made on the basis of water content, not of volume. If the inital value of the 2:1 diffusion potential was less than 8.2 mV, the gall-bladder was discarded. Under these conditions of asymmetrical bathing solutions, the junction potential arising at the agar–NaCl

bridges was calculated from a modified Henderson formula:

$$\Delta E = -\frac{(u_+ - u_-)}{(u_+ + u_-)} \frac{RT}{F} \ln \frac{a''}{a'}$$

[*see* MacInnes (1961) and Barry and Diamond *(in preparation)* for further discussion of the complex problem of junction potentials] and subtracted from the experimental p.d. In all other situations, the mucosal and serosal solutions were identical in composition, and there was no net junction potential in the circuit.

Temperatures other than ambient (23 ± 1 °C) were obtained by conducting the experiment in a water bath at 37 ± 0.5 °C or in an ice bath at 4 ± 1 °C.

The rate of fluid transport was measured gravimetrically, as described previously (Diamond, 1962*a*, 1964*a*).

Table 1 gives the composition of experimental solutions. In the text, solution A is referred to as NaCl Ringer's solution, C as NaCl–$NaHCO_3$ Ringer's solution, E as Na_2SO_4 Ringer's solution, F as choline Ringer's, and G as Na_2SO_4–$NaHCO_3$ Ringer's solution. All solutions had a pH of 7.3 ± 0.1 as checked with a glass electrode. Solutions A–G were 274 ± 8 milliosmolal as measured with a Fiske osmometer; solution H had a higher osmolality due to the added sucrose. For metabolic inhibition of the gall-bladder, an isotonic stock solution of 103 mM NaCN plus 103 mM iodoacetic acid was added to solution C to give the desired final concentration of CN^- and iodoacetate (1 or 3 mM). Solutions made hypertonic with sucrose to determine the osmotic gradient necessary to stop fluid transport were obtained by mixing solutions C and H.

All errors are reported as standard deviations.

Results

Effect of Temperature

Maximal rates of fluid transport by the gall-bladder are observed when the temperature is 37 °C and the bathing solutions contain 25 mM bicarbonate in addition to NaCl as the principal salt (Diamond, 1964*a*). Hence, the p.d. was measured in 33 gall-bladders at 37 °C, using NaCl–$NaHCO_3$ Ringer's solution (solution C, Table 1) as both the mucosal and the serosal bathing solution. The average value and S. D. of the p.d. were $+1.35 \pm 0.35$ mV (i.e., mucosal-solution positive). The highest value was +2.40 mV, and the lowest +0.70 mV.

Since the fluid transport rate decreases with decreasing temperature, the p.d. was measured alternately at 4 and 37 °C in 24 gall-bladders bathed in NaCl–$NaHCO_3$ Ringer's solution. In every case, the p.d. decreased on going from 37 to 4 °C and returned to approximately the original value on going from 4 back to 37 °C; this reversible effect could be elicited repeatedly in the same gall-bladder. Fig. 2 illustrates this effect for one gall-bladder, and Tables 2–7 illustrate it for 15 others. The average value and S.D. of the p.d. at 4 °C were $+0.20 \pm 0.10$ mV (24 gall-bladders), and all values fell between 0.00 and 0.40 mV. In three additional gall-bladders, cooling

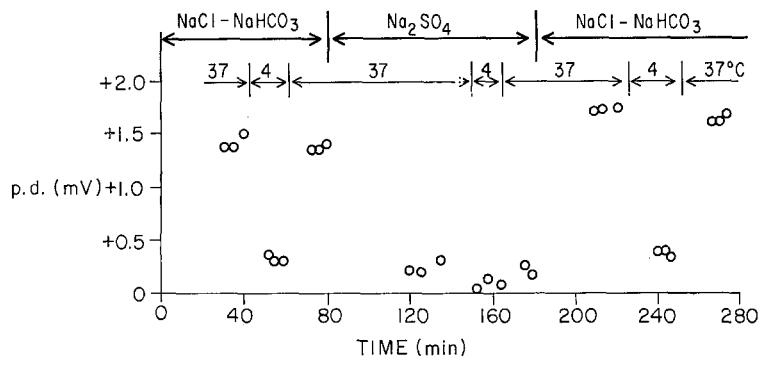

Fig. 2. An example of experimental protocol B. The ordinate gives the electrical p.d. across a rabbit gall-bladder, expressed as the potential of the mucosal solution with respect to that of the serosal solution. The bathing solution was initially NaCl–$NaHCO_3$ Ringer's solution (solution C, Table 1), was changed to Na_2SO_4 Ringer's solution (solution E, Table 1) at 80 min, and was changed back to NaCl–$NaHCO_3$ Ringer's solution at 180 min. At any given time, the same solution served both as the mucosal and the serosal bathing solution. The temperature was alternately 37 and 4 °C, as indicated by the arrows. Note that the p.d. in Na_2SO_4 Ringer's solution is near zero at either temperature, but that in NaCl–$NaHCO_3$ Ringer's solution it increases at 37 °C to about 1.6 mV; the effects of changing solutions or temperature are reversible and repeatable

from 37 to 23 °C was found to reduce the p.d. reversibly but by less than did cooling to 4 °C – from $+1.80 \pm 0.45$ to $+0.80 \pm 0.30$ mV.

This p.d. in maximally transporting gall-bladders is in the direction expected (mucosal-solution positive) for a diffusion potential resulting from locally raised NaCl concentrations in the lateral intercellular spaces. The five series of experiments to be reported next show that this p.d. is abolished or greatly reduced by five sets of conditions which abolish or greatly reduce fluid transport (replacement of chloride with sulfate, replacement of chloride and bicarbonate with sulfate, replacement of sodium with choline, removal of bicarbonate, and metabolic poisoning). Since the magnitude of the p.d. even in maximally transporting gall-bladders is small, all experiments were designed to use each gall-bladder as its own control, by means of either of two protocols: *Protocol A.* At 37 °C the p.d. was repeatedly and alternately measured in NaCl–$NaHCO_3$ Ringer's solution and in a solution which inhibits transport (Fig. 3). *Protocol B.* The effect on the p.d. of cooling from 37 to 4 °C and rewarming from 4 to 37 °C was measured in NaCl–$NaHCO_3$ Ringer's solution, then in a solution which inhibits transport, and finally in NaCl–$NaHCO_3$ Ringer's solution again (Fig. 2). The purpose of these "bracketed" experimental designs was to make certain that the effects studied were real and reproducible despite their small size.

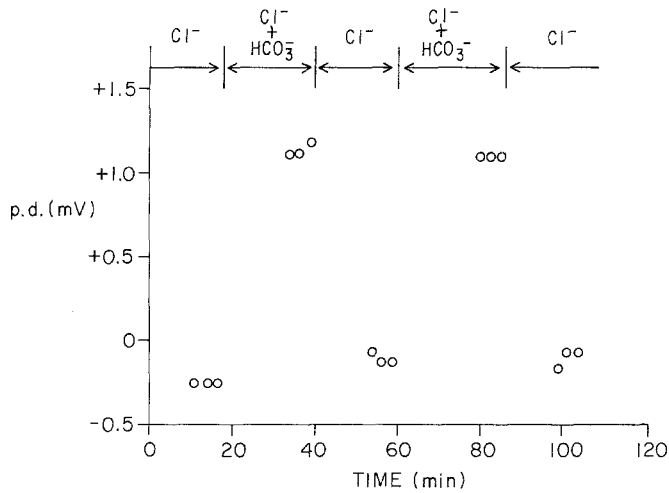

Fig. 3. An example of experimental protocol A. The ordinate gives the electrical p.d. across a rabbit gall-bladder, expressed as the potential of the mucosal solution with respect to that of the serosal solution. The temperature was kept constant at 37 °C, and the bathing solution was repeatedly changed back and forth between NaCl Ringer's solution (solution A, Table 1) and NaCl–$NaHCO_3$ Ringer's solution (solution C, Table 1), as indicated by the arrows. At any given time, the same solution served as both the mucosal and the serosal bathing solution. Note that removal of HCO_3^- reversibly and repeatedly abolishes the p.d.

Effect of Replacing Cl^- and HCO_3^- by SO_4^{--}

Since the NaCl pump in the gall-bladder shows anion specificity, replacement of NaCl and $NaHCO_3$ by Na_2SO_4 greatly reduces or abolishes fluid transport in the gall-bladder (Diamond, 1962*a*; Dietschy, 1964; Martin & Diamond, 1966; Whitlock & Wheeler, 1967). The effect of this replacement on the p.d. was tested by means of protocol B in three gall-bladders. As shown in Fig. 2 and Table 2, replacement of NaCl and $NaHCO_3$ with Na_2SO_4 reversibly reduces or abolishes both the p.d. observed at 37 °C and the decrease in p.d. observed on cooling in NaCl–$NaHCO_3$ Ringer's solution. At 37 °C the average p.d. was $+0.25 \pm 0.30$ mV in Na_2SO_4 Ringer's solution, but it was $+1.35 \pm 0.35$ mV in NaCl–$NaHCO_3$ Ringer's solution in the same three gall-bladders.

Effect of Replacing Cl^- by SO_4^{--}

Bicarbonate stimulates NaCl transport (Diamond, 1964*a*; Wheeler, Ross, & King, 1969), and is actively transported itself but much less efficiently than Cl^- (Wheeler, 1963; Diamond, 1964*b*). To be certain that the

Table 2. *Effect of replacing Cl^- and HCO_3^- by $SO_4^=$* [a]

Experiment no.	Solution								
	$NaCl$–$NaHCO_3$			Na_2SO_4			$NaCl$–$NaHCO_3$		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
28	+1.75	+0.20	+1.45	−0.25	+0.50	+0.60	+1.30	+0.20	+1.40
29	+1.50	+0.30	+1.40	+0.30	+0.20	+0.20	+1.80	+0.35	+1.75
30	+1.10	+0.15	+1.20	+0.40	+0.20	+0.35	+0.75	+0.10	+0.80

[a] The table gives the p.d. across the gall-bladder in mV (potential of mucosal solution with respect to serosal solution). In each of three gall-bladders, the p.d. was measured in the indicated sequence of solution (*see* Table 1 for composition) and at the indicated temperatures. During each measurement the indicated solution served as both the mucosal and the serosal bathing solutions. Note that the p.d. at 37 °C is higher in $NaCl$–$NaHCO_3$ than in Na_2SO_4, that cooling decreases the p.d. in $NaCl$–$NaHCO_3$ but only slightly or not at all in Na_2SO_4, and that the effects are small but reversible and repeatable.

Table 3. *Effect of replacing Cl^- by $SO_4^=$* [a]

ri-ent.	Solution								
	$NaCl$–$NaHCO_3$			Na_2SO_4–$NaHCO_3$			$NaCl$–$NaHCO_3$		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
	+1.30	+0.25	+1.05	−0.25	−0.05	+0.10	+0.95	+0.10	+1.30

ri-ent.	Solution				
	$NaCl$–$NaHCO_3$	Na_2SO_4–$NaHCO_3$	$NaCl$–$NaHCO_3$	Na_2SO_4–$NaHCO_3$	NaCl-$NaHCO_3$
	37 °C	37 °C	37 °C	37 °C	37 °C
	+1.70	+0.25	+1.50	+0.30	+2.10
	+0.90	−0.10	+0.60	−0.20	+0.60

[a] The table gives the p.d. across the gall-bladder in mV, for the indicated sequence of solutions (used as both mucosal and serosal bathing solution) and the indicated temperatures, in each of three gall-bladders. Note that the p.d. at 37 °C is higher in $NaCl$–$NaHCO_3$ than in Na_2SO_4–$NaHCO_3$, that this difference is greatly reduced on cooling, and that the effects are reversible and repeatable.

p.d. in $NaCl$–$NaHCO_3$ Ringer's solution at 37 °C is mainly due to NaCl transport rather than associated somehow with HCO_3^- transport itself. The effect of replacing NaCl with Na_2SO_4 while leaving $NaHCO_3$ in the bathing solution was tested in two gall-bladders by protocol A and in one gall-bladder by protocol B. As summarized in Table 3, replacement of NaCl with Na_2SO_4 still reversibly abolishes or greatly reduces the p.d. even when HCO_3^- is present. At 37 °C the average p.d. was 0.00 ± 0.25 mV in Na_2SO_4–

$NaHCO_3$ Ringer's solution, but it was $+1.20 \pm 0.50$ mV in NaCl–$NaHCO_3$ Ringer's solution in the same three gall-bladders. Hence, the latter p.d. cannot be directly due to HCO_3^-.

Effect of Replacing Na^+ by Choline

Since the NaCl pump in the gall-bladder shows cation as well as anion specificity, replacement of NaCl and $NaHCO_3$ by choline chloride and choline bicarbonate abolishes fluid transport (Wheeler, 1963; Dietschy, 1964). In three experiments based on protocol B and summarized in Table 4,

Table 4. *Effect of replacing Na^+ by choline$^+$* [a]

Experiment no.	Solution					
	Choline			NaCl–$NaHCO_3$		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
15	+0.35	+0.10	+0.25	+1.00	0.00	+1.55
16	+0.30	+0.05	+0.30	+1.35	+0.25	+1.30
17	+0.10	+0.05	+0.15	+0.80	+0.15	+0.70

[a] The table gives the p.d. across the gall-bladder in mV, for the indicated sequence of solutions (used as both mucosal and serosal bathing solutions) and the indicated temperatures, in each of three gall-bladders. Note that the p.d. at 37 °C is higher in NaCl–$NaHCO_3$ than in choline and that this difference is greatly reduced on cooling.

replacement of all Na^+ with choline reversibly reduced the p.d. at 37 °C and the change in p.d. observed on cooling. Since relatively long times were found to be required for the slowly diffusing choline cation to replace Na^+ in the gall-bladder wall, the protocol was shortened to a single set of measurements in NaCl–$NaHCO_3$ preceded by the set in choline. At 37 °C the average p.d. was $+0.25 \pm 0.10$ mV in choline Ringer's solution, but it was $+1.10 \pm 0.35$ mV in NaCl–$NaHCO_3$ Ringer's solution in the same three gall-bladders.

Effect of Replacing HCO_3^- by Cl^-

Since HCO_3^- stimulates NaCl transport in the gall-baldder, the transport rate is lower in bicarbonate-free solutions than in solutions containing 25 mM HCO_3^- in addition to NaCl as the main salt. In three experiments based on protocol A and one experiment based on protocol B (Fig. 3, Table 5), replacement of the 25 mM HCO_3^- in NaCl–$NaHCO_3$ Ringer's

Table 5. *Effect of replacing HCO_3^- by Cl^-* [a]

Experiment no.	Solution								
	$NaCl$–$NaHCO_3$			$NaCl$			$NaCl$–$NaHCO_3$		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
31	+1.30	+0.25	+1.40	+0.15	+0.05	+0.25	+1.40	+0.05	+1.45

Experiment no.	Solution				
	$NaCl$	$NaCl$–$NaHCO_3$	$NaCl$	$NaCl$–$NaHCO_3$	$NaCl$
	37 °C	37 °C	37 °C	37 °C	37 °C
32	−0.05	+1.20	+0.10	+1.05	+0.10
33	−0.10	+0.70	0.00	+0.80	0.00
34	−0.25	+1.20	−0.10	+1.10	−0.05

[a] The table gives the p.d. across the gall-bladder in mV, for the indicated sequence of solutions (used both as mucosal and serosal bathing solution) and the indicated temperatures, in each of four gall-bladders. Note that the p.d. at 37 °C is higher in $NaCl$–$NaHCO_3$ than in NaCl and that this difference is greatly reduced on cooling.

solution with Cl^- while leaving $[Na^+]$ unchanged reversibly abolished the p.d. at 37 °C and the change in p.d. on cooling. At 37 °C the average p.d. in NaCl Ringer's solution was 0.00 ± 0.15 mV, but it was $+1.15 \pm 0.25$ mV in $NaCl$–$NaHCO_3$ Ringer's solution in the same four gall-bladders.

The same conclusion emerges from a comparison at 23 °C in three gall-bladders by means of protocol A. The p.d. was $+0.80 \pm 0.30$ mV in $NaCl$–$NaHCO_3$ Ringer's solution but reversibly decreased to -0.25 ± 0.10 mV on replacement of the $NaHCO_3$ with NaCl. The p.d. at 23 °C in NaCl Ringer's solution (HCO_3^--free) was routinely measured in all 42 gall-bladders used in this study, and averaged -0.20 ± 0.15 mV.

Effect of Metabolic Poisoning

The combination of cyanide and iodoacetate abolishes fluid transport by the gall-bladder (Diamond, 1962*a*, 1964*a*; Tormey & Diamond, 1967). As seen in Table 6, which summarizes four experiments by means of protocol B (two experiments using 3 mM CN^- and 3 mM iodoacetate, two using 1 mM CN^- and 1 mM iodoacetate), metabolic poisoning abolishes the p.d. observed at 37 °C. The first measurement at 37 °C after exposure to the poisons always yielded a higher p.d. than the second, presumably because inhibition was not yet complete at the time of the first measurement. The average value of the second measurement was $+0.20 \pm 0.05$ mV, com-

Table 6. *Effect of metabolic poisoning* [a]

Experiment no.	Inhibitor concentration					
	0			3 mM		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
24	+1.85	+0.15	+1.85	+0.30	+0.20	+0.15
25	+1.20	+0.30	+1.10	+0.50	+0.10	+0.15

Experiment no.	Inhibitor concentration					
	0			1 mM		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
26	+2.10	+0.40	+1.65	+0.85	+0.35	+0.30
27	+1.25	+0.35	+1.30	+0.65	+0.30	+0.20

[a] Both the mucosal and serosal solutions were NaCl–$NaHCO_3$ Ringer's solution, to which had been added no inhibitor, or 1 mM CN^- plus 1 mM iodoacetate, or 3 mM CN^- plus 3 mM iodoacetate. The table gives the p.d. across the gall-bladder in mV, for the indicated sequence of solutions and temperatures, in each of four gall-bladders. Note that the p.d. in NaCl–$NaHCO_3$ is higher at 37 °C than at 4 °C, and that this is reduced and eventually abolished by metabolic poisoning.

pared to $+1.55 \pm 0.35$ mV before poisoning in the same gall-bladders. Since previous experience had indicated that the effect of metabolic poisoning on fluid transport in the gall-bladder is poorly reversible, no attempt was made to wash away the poisons and restore the original p.d.

Effect of Partial Replacement of NaCl by Sucrose

The final absorbate formed by the gall-bladder always has the same osmolarity as the bathing solution, whatever the latter's value (Diamond, 1964*b*). Thus, if the main solute in the bathing solution is NaCl, the Na^+ concentration in the absorbate and the bathing solution are virtually identical; but if the bathing solution contains the impermeant nonelectrolyte sucrose in addition to NaCl, the salt concentration in the absorbate is higher than in the bathing solution, since osmotic equilibration yields an extra 1 mM NaCl in the absorbate for every 2 mM sucrose in the bathing solution. Thus, one would expect higher p.d. after partial replacement of bathing solution NaCl with sucrose, since the excess of [NaCl] in the lateral spaces over [NaCl] in the bathing solutions would now have to be large enough to replace sucrose osmotically as well as to make the lateral spaces hypertonic.

Table 7. *Effect of partial replacement of NaCl by sucrose*[a]

Experiment no.	NaCl concentration								
	110 mM			60 mM			110 mM		
	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C	37 °C	4 °C	37 °C
20	+1.35	+0.10	+1.20	+3.20	+0.45	+3.90	+0.55	+0.05	+0.90
21	+1.00	+0.10	+0.95	+2.40	+0.40	+2.20	+0.80	+0.10	+0.75
22	+1.10	+0.20	+1.20	+2.65	+0.35	+2.70	+0.50	+0.10	+0.50

[a] The table gives the p.d. across the gall-bladder in mV, for the indicated sequence of solutions (used as both mucosal and serosal bathing solutions) and indicated temperatures, in each of three gall-bladders. The bathing solution was either NaCl–$NaHCO_3$ Ringer's solution, [NaCl] 110 mM, no sucrose, or an otherwise identical solution in which NaCl had been partially replaced isosmotically with sucrose to lower [NaCl] to 60 mM (solution D, Table 1). Note that at 37 °C the p.d. is higher in 60 mM NaCl than in 110 mM NaCl and that these p.d. are reduced by cooling.

This prediction was confirmed in three gall-bladders by means of protocol B (Table 7). Isosmotic replacement of 50 mM NaCl with sucrose (solution D, Table 1) reversibly increased the p.d. at 37 °C and the change in p.d. on cooling. At 37 °C the average p.d. was $+2.85 \pm 0.60$ mV after this replacement, but it was $+0.90 \pm 0.30$ mV in NaCl–$NaHCO_3$ Ringer's solution in the same gall-bladders. Whitlock and Wheeler (1964) observed a similar increase in p.d. in NaCl–sucrose mixtures and similarly interpreted it in terms of a raised NaCl concentration at the site of local osmotic equilibration.

Effect of Temperature on NaCl Diffusion Potentials

At the beginning and end of each experiment, we routinely measured the diffusion potential resulting at 23 °C from replacing half the [NaCl] in the mucosal solution with mannitol. In five experiments, we compared the diffusion potential resulting from this same 2:1 gradient at 37, 23, and 4 °C, in order to be able to correct the diffusion potentials routinely measured at 23 to 37 °C for calculating concentrations in the lateral spaces at 37 °C from the p.d. Increasing temperature was found to increase reversibly the 2:1 diffusion potential. The average value of this diffusion potential for these five gall-bladders was 5.40 ± 1.85 mV at 4 °C, 8.15 ± 1.70 mV at 23 °C, and 11.40 ± 1.75 mV at 37 °C (dilute-solution positive in all cases). This change is greater than that expected from the factor RT/F in the Nernst or constant-field equations (*see* p. 206), and implies that sodium permeability increases more rapidly than chloride permeability with increasing temperature. However, the 53% decrease in the 2:1 NaCl diffusion potential from

37 to 4 °C is still much less than the 86% decrease over the same temperature range in the p.d. measured with NaCl-$NaHCO_3$ Ringer's solution as both the mucosal and serosal bathing solution. Thus, the effect of temperature in the latter case cannot be attributed to the change in relative permeability coefficients with temperature but must reflect mainly a smaller local concentration gradient in the lateral spaces at lower temperatures, owing to a lower rate of NaCl pumping.

The dependence of diffusion potentials in the gall-bladder at constant temperature on ion concentration gradients is found experimentally to be approximately represented by the constant-field equation:

$$\Delta E = \frac{RT}{F} \ln \frac{P_{Na}\gamma_s[Na]_s + P_K\gamma_s[K]_s + P_{Cl}\gamma_m[Cl]_m}{P_{Na}\gamma_m[Na]_m + P_K\gamma_m[K]_m + P_{Cl}\gamma_s[Cl]_s} \quad (1)$$

where ΔE is the electrical potential of the mucosal solution with respect to the serosal solution; R, the gas constant; T, absolute temperature; F, the Faraday; P, a permeability coefficient; γ, an activity coefficient; and subscripts s and m refer to the serosal and mucosal solutions, respectively. P_K/P_{Na} for rabbit gall-bladder is 2.2 (Wright & Diamond, 1968; P. H. Barry, *personal communication*). Activity coefficients were taken from Robinson and Stokes (1965), assuming $\gamma_{Na} = \gamma_K = \gamma_{Cl}$ in any given solution.

The average value of the 2:1 NaCl diffusion potential at 23 °C for all 41 gall-bladders used in this study was 9.70 ± 1.25 mV, and the average value of P_{Cl}/P_{Na} calculated from Eq. (1) was 0.15 ± 0.07. For the five gall-bladders in which diffusion potentials were measured as a function of temperature, P_{Cl}/P_{Na} at 37 °C was on the average 47% of the value at 23 °C in the same gall-bladder.

Effect of Imposed Osmotic Gradients on Fluid Transport

When the osmolarities of the mucosal and serosal bathing solutions are identical, active NaCl transport in the gall-bladder generates a mucosa-to-serosa water flux. If the mucosal solution is made progressively hypertonic by addition of the impermeant nonelectrolyte sucrose, the mucosa-to-serosa water flux progressively decreases; it becomes zero for mucosal sucrose concentrations of about 80 to 100 mM (Wheeler, 1963; Diamond, 1964*a*; Dietschy, 1964; Whitlock & Wheeler, 1964), and it reverses (net water flux becomes serosa-to-mucosa) for higher sucrose concentrations. As shown in the Discussion, the mucosal hypertonicity required to bring net fluid transport to zero provides an independent means of estimating the salt concentration in the lateral spaces under zero-flow conditions;

this estimate can be compared with the estimate derived from the p.d. in this situation. Hence, the fluid transport rate was measured gravimetrically as a function of imposed osmotic gradients in four gall-bladders at 37 °C (using solution C, Table 1, as the serosal solution, and mixtures of solutions C and H in varying proportions as the mucosal solution). This experimental procedure is identical to that described and illustrated previously (Fig. 4; Diamond, 1964*a*).

When NaCl–$NaHCO_3$ Ringer's solution was used both as the mucosal and serosal bathing solution, so that there was no osmotic gradient between the external bathing solutions, the average rate of mucosa-to-serosa fluid transport was 84 ± 24 μliters/hr, cm^2, which is comparable to the average rates obtained in vitro for rabbit gall-bladder by previous workers (Wheeler, 1963; Diamond, 1964*a*; Dietschy, 1964; Tormey & Diamond, 1967). The added mucosal sucrose concentration required to bring fluid movement to zero averaged 95 ± 22 mM, in agreement with the values of 80 to 100 mM obtained by previous workers.

In three gall-bladders, the p.d. was measured in NaCl–$NaHCO_3$ Ringer's solution after 100 mM sucrose had been added to the mucosal solution to approximate a condition of zero flow. Upon addition of 100 mM sucrose, the p.d. rose promptly from $+1.25 \pm 0.35$ mV to $+6.70 \pm 0.90$ mV. This p.d. in the presence of an external osmotic gradient was formerly interpreted as an electrokinetic flow potential or streaming potential (Diamond, 1962*c*; Dietschy, 1964; Diamond & Harrison, 1966). However, it has recently been shown that most or all of this streaming potential is actually a boundary diffusion potential due to the NaCl concentration difference between the lateral spaces and the mucosal bathing solution (Wedner & Diamond, 1969), just as is the smaller p.d. of ca. 1.25 mV under maximal transport conditions in the absence of an external osmotic gradient. After this initial prompt rise to 6.7 mV, the p.d. slowly rose further over the course of an hour to stabilize at a final value of 10 to 12 mV. This slow increase is due to a slow build-up in the NaCl concentration of the luminal bathing solution, since mucosa-to-serosa active salt transport continues even though water transport has been brought to zero. The luminal [NaCl], hence the p.d., eventually stabilizes at a level where mucosa-to-serosa active NaCl transport is just balanced by serosa-to-mucosa diffusion of NaCl down its concentration gradient from the concentrated luminal solution. The correctness of this interpretation is shown by the fact that replacement of the luminal solution with fresh NaCl–$NaHCO_3$ Ringer's solution caused the p.d. to drop back to near 6.7 mV, after which it would slowly begin to rise again.

Discussion

Origin of the p.d.

Most epithelia (frog skin, urinary bladder, stomach, etc.) develop p.d. of up to 150 mV when separating identical bathing solutions. These p.d. result from the presence of pumps which transfer cations not anions (or vice versa), and which are coupled electrically rather than directly to anion (or cation) movement. In contrast, all studies of transport in the gall-bladder have demonstrated that the p.d. between identical solutions is close to zero. It may be concluded from a quantitative analysis (Diamond, 1962*b*, 1968; *see also* Wheeler, 1963, and Dietschy, 1964) that both Na^+ and Cl^- are actively transported in 1:1 proportions by an electrically neutral NaCl pump. The present study shows that the p.d. across maximally transporting rabbit gall-bladders averages $+1.35 \pm 0.35$ mV (33 gall-bladders), a value which would be considered trivial and equated with zero in most other epithelia. However, this small value differs significantly from zero and is somehow associated with fluid transport, since it can be repeatedly and reversibly reduced or abolished by any one of six procedures tested which reduce or abolish fluid transport.

This small mucosa-positive p.d. cannot be considered a direct expression of ion pumps, as in other epithelia, because both Na^+ and Cl^- are actively transported by the gall-bladder in the mucosa-to-serosa direction against large electrochemical gradients. To attribute the p.d. directly to pumping, one would have to assume that the cation and anion pumps were independent of each other and that their oppositely oriented p.d. nearly cancelled each other, but that the anion pump operated slightly faster, leaving the mucosal solution slightly positive electrically. In such a case, the p.d. should have increased in choline Ringer's solution, a situation in which the cation pump would be inoperative and only the anion pump active; the predicted value due to an independent anion pump in choline Ringer's solution is 45 mV (*see* Diamond, 1962, p. 497, & 1968, p. 2470, for details of this and of the corresponding calculation eliminating independent cation pumps). In reality, not only did the p.d. fail to increase to these large values in choline Ringer's solution (or in tetraethylammonium Ringer's solution; Diamond, 1962*b*), but it actually decreased to $+0.25$ mV, showing that no electrogenic anion pump exists in the gall-bladder. Thus, the cation and anion pumps must be tightly coupled to each other, inoperative in each other's absence, and hence incapable of directly producing a p.d.

The sign of the small p.d. in maximally transporting rabbit gall-bladders is in fact that of the expected local diffusion potential resulting from the

facts that [NaCl] in the lateral spaces is higher than in the mucosal bathing solution and that the gall-bladder is more permeable to cations than to anions. The observed increase in the p.d. when NaCl in the bathing solutions is partially replaced with sucrose also conforms to the expected behavior for a local diffusion potential, since this replacement will increase the salt gradient between the lateral spaces and the mucosal solution. Whitlock and Wheeler (1964) noticed that the p.d. in maximally transporting rabbit gall-bladders was very small but consistently mucosa-positive and that it increased with partial sucrose substitution. They pointed out that these p.d. were expected from considerations of a local osmotic compartment and the preferential cation permeability of the gall-bladder.

Estimation of the Salt Concentration in the Lateral Spaces

Since a 2:1 NaCl diffusion potential was measured in each experiment and the salt gradient corresponding to any other given p.d. in the same gall-bladder may thus be calculated, the small p.d. observed in maximally transporting gall-bladders (NaCl–$NaHCO_3$ Ringer's solution, 37 °C) yields an estimate of the mean salt concentration in the lateral spaces during transport. For the purposes of calculation, the following assumptions are made:

(a) Na^+, Cl^-, K^+, and HCO_3^- are assumed to be the only ions present in the lateral spaces during maximal fluid transport. The other four ions in the bathing solution ([Ca^{++}] 1.0 mM, [Mg^{++}] 1.2 mM, [SO_4^{--}] 1.2 mM, [$H_2PO_4^-$] 1.2 mM) are probably less permeant than Na^+, K^+, or Cl^-, and are present at such low concentrations that they could hardly affect the p.d. Thus, the p.d. should be approximated by the constant-field equation with terms for only the four major ions, using the subscript *l* to refer to the lateral spaces:

$$\Delta E = \frac{RT}{F} \ln \frac{P_{Na}\gamma_l[Na]_l + P_K\gamma_l[K]_l + P_{Cl}\gamma_m[Cl]_m + P_{HCO_3}\gamma_m[HCO_3]_m}{P_{Na}\gamma_m[Na]_m + P_K\gamma_m[K]_m + P_{Cl}\gamma_l[Cl]_l + P_{HCO_3}\gamma_l[HCO_3]_l}. \quad (2)$$

(b) $[K^+]_l$ is assumed approximately equal to $[K^+]_m$, the bathing-solution value of 7 mM, on the basis of measurements of absorbate K^+ concentration (Diamond, 1964*b*; Fig. 5).

(c) $[HCO_3^-]_l$ is assumed equal to 18 mM, since this is the value found for $[HCO_3^-]$ in the absorbate when $[HCO_3^-]$ is 25 mM in the bathing solutions (Diamond, 1964*b*). Since the relation $[Na^+]_l + [K^+]_l = [Cl^-]_l + [HCO_3^-]_l$ follows from the electroneutrality condition, and since $[K^+]_l = 7$ and $[HCO_3^-]_l = 18$, one obtains $[Cl^-]_l = [Na^+]_l - 11$.

The value of P_{Cl}/P_{Na} calculated from the 2:1 NaCl diffusion potential at 23 °C in each gall-bladder was multiplied by 0.47 to correct it to 37 °C (*see* Results). Since the value of P_{Cl}/P_{Na} was generally found to increase slowly with time in a gall-bladder, the value corresponding to the time at which we measured the p.d. in NaCl–$NaHCO_3$ Ringer's solution at 37 °C was calculated by linear interpolation with time between the values measured at the beginning and end of each experiment. Into Eq. (2) were then inserted the ion concentrations in the mucosal bathing solution ($[Na^+]_m$ 136.2, $[K^+]_m$ 7, $[Cl^-]_m$ 119, $[HCO_3^-]_m$ 25; $[K^+]_l = 7$, $[Cl^-]_l = [Na^+]_l - 11$, $[HCO_3^-]_l$ 18). Also inserted were the estimated activity coefficients from Robinson and Stokes (1965) (γ_m 0.754, γ_l 0.750 to 0.746, depending on the calculated value of $[Na^+]_l$); P_K/P_{Na} 2.2; and the value of the p.d. in NaCl–$NaHCO_3$ Ringer's solution, together with the value of P_{Cl}/P_{Na}; for each gall-bladder.

Eq. (2) was then solved for each gall-bladder to obtain $[Na^+]_l$. Since P_{HCO_3} was not known experimentally but presumably falls somewhere between 0 and P_{Cl}, two sets of calculations were carried out, for the cases $P_{HCO_3} = 0$ and $P_{HCO_3} = P_{Cl}$. The former assumption yielded $[Na^+]_l = 148.8 \pm 3.3$ and $[Cl^-]_l = 137.7 \pm 3.4$ mM (average values and S.D. for 30 gall-bladders). The latter assumption yielded $[Na^+]_l = 147.6 \pm 3.6$, $[Cl^-]_l = 136.6 \pm 3.6$ mM. Thus, the mean sodium concentration, or mean value of $[Cl^-]$ plus $[HCO_3^-]$, in the lateral spaces is estimated to be about 12 mM above the bathing solution values. This first estimate requires a small correction owing to the fact that raised salt concentrations in the lateral spaces not only set up a transepithelial diffusion potential, because of P_{Na} exceeding P_{Cl}, but it also must establish a junction potential down the lateral spaces with the same sign as the diffusion potential and in series with it, because Cl^- has a higher transport number than Na^+ in free solution. Subtraction of this estimated junction potential from the total measured potential reduces the estimate of the mean excess salt concentration from 12 to 10 mM. Since 1 mM NaCl represents 2 mM osmotically active solute, this indicates that the mean amount by which the lateral spaces are hypertonic to the bathing solution is about $2 \times 10 = 20$ mosm (neglecting osmotic coefficients and the unknown small gradients of the minor constituents of Ringer's solution).

Because this method of estimating the salt concentration in the lateral spaces during maximal transport is an indirect one and subject to several sources of uncertainty (next paragraph), it would be desirable to have some situation in which this electrically estimated concentration can be compared with an estimate obtained by an independent method. Such a comparison is possible in the situation where a sufficient concentration of sucrose has

been added to the mucosal solution to bring the net water flux to zero. Under these conditions of zero net water flux, the mean osmolality in the lateral spaces must equal that in the mucosal solution. Zero water flow was found to occur at a mucosal sucrose concentration of about 95 mM (*see* Results); therefore, [NaCl] in the lateral spaces must be about 47.5 mM (or 51 mM after correction for osmotic coefficients) higher than that in the mucosal solution under zero-flow conditions. Substitution of the p.d. measured under these circumstances in three gall-bladders ($+6.70 \pm 0.90$ mV) into Eq. (2) yields average values of $[Na^+]_l$ 198 ± 3 and $[Cl^-]_l$ 187 ± 3 mM if $P_{HCO_3} = 0$ or if $P_{HCO_3} = P_{Cl}$. Thus, the mean salt concentration (mean value of $[Na^+]$ or of $[Cl^-]$ plus $[HCO_3^-]$) in the lateral spaces during zero water flow would be to a first approximation about 62 mM above the bathing solution values; correction for the small junction potential in the lateral spaces reduces this figure to 48 mM. This estimate of 48 mM derived from the electrical method is in good agreement with the value of 51 mM obtained from the zero-flow sucrose concentration, and provides independent support for the validity of using the electrical method to estimate concentrations during maximal water flow. Since net water flow into the lateral spaces would dilute their NaCl concentration, it is reasonable and to be expected that the estimated excess concentration of 48 to 51 mM during zero flow should exceed the value of 10 mM estimated under maximal flow.

Sources of Uncertainty

Three factors must be borne in mind in regard to the estimate that the mean salt concentration in the lateral spaces is about 10 mM higher than in the bathing solutions.

First, the geometry of the lateral spaces (long, narrow, dead-end, unstirred channels) makes it highly unlikely that the salt concentration is uniform throughout them. Instead, standing osmotic and concentration gradients will be present, such that the salt concentration decreases from maximally hypertonic at the closed end to isotonic at the open end (Diamond & Bossert, 1967, 1968). The p.d. will vary similarly with distance, so that the measured p.d., hence the estimated salt concentration, is a mean value for the whole length of the spaces. In particular, values of salt concentration more than 10 mM above bathing solution values are to be expected toward the closed ends of the spaces.

Second, the details of salt and water movement from the mucosal bathing solution into the cell are still unclear, and the speculative possibility exists that standing gradients are established in the microvilli of the mucosal

membrane as well as in the lateral spaces (Diamond & Bossert, 1967, 1968). If this were the case, part of the total mean gradient of 10 mM would be in the microvilli, the remainder in the lateral spaces.

Finally, a p.d. in the lateral spaces, with a total membrane area of about 550 square μ per cell (neglecting increase in area due to cytoplasmic projections and folding) will be to some extent shunted by the flat serosal face of the cell (adjacent to the basement membrane), with an area of about 20 square μ per cell and across which transport or a p.d. associated with transport is not expected to occur.

Because of these sources of uncertainty, the estimate of the excess salt concentration in the lateral spaces as 10 mM should be regarded as indicating only an order of magnitude. Direct microanatomical demonstration of this small a difference will require a quantitative and careful study.

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References

Curran, P. F., and A. K. Solomon. 1957. Ion and water fluxes in the ileum of rats. *J. Gen. Physiol.* **41**:143.

Diamond, J. M. 1962*a*. The reabsorptive function of the gall-bladder. *J. Physiol.* **161**:442.

— 1962*b*. The mechanism of solute transport by the gall-bladder. *J. Physiol.* **161**:474.

— 1962*c*. The mechanism of water transport by the gall-bladder. *J. Physiol.* **161**:503.

— 1964*a*. Transport of salt and water in rabbit and guinea pig gall-bladder. *J. Gen. Physiol.* **48**:1.

— 1964*b*. The mechanism of isotonic water transport. *J. Gen. Physiol.* **48**:15.

— 1965. The mechanism of isotonic water absorption and secretion. *In* The State and Movement of Water in Living Organisms, XIXth Symp. Soc. Exptl. Biol. p. 329. Cambridge Univ. Press, Cambridge.

— 1968. Transport mechanisms in the gall-bladder. *In* Handbook of Physiology: Alimentary Canal, vol. 5. p. 2451. American Physiological Society, Washington.

—, and W. H. Bossert. 1967. Standing-gradient osmotic flow: a mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* **50**:2061.

— — 1968. Functional consequences of ultrastructural geometry in "backwards" fluid-transporting epithelia. *J. Cell Biol.* **37**:694.

—, and S. C. Harrison. 1966. The effect of membrane fixed charges on diffusion potentials and streaming potentials. *J. Physiol.* **183**:37.

—, and J. M. Tormey. 1966. Role of long extracellular channels in fluid transport across epithelia. *Nature* **210**:817.

Dietschy, J. M. 1964. Water and solute movement across the wall of the everted rabbit gall bladder. *Gastroenterology* **47**:395.

Kaye, G. I., H. O. Wheeler, R. T. Whitlock, and N. Lane. 1966. Fluid transport in the rabbit gall bladder. *J. Cell Biol.* **30**:237.

MacInnes, D. A. 1961. The Principles of Electrochemistry. Dover Publications, Inc., New York.

Martin, D. W., and J. M. Diamond. 1966. Energetics of coupled active transport of sodium and chloride. *J. Gen. Physiol.* **50**:295.

Ogilvie, J. T., J. R. McIntosh, and P. F. Curran. Volume flow in a series-membrane system. *Biochim. Biophys. Acta* **66**:441.

Robinson, R. A., and R. H. Stokes. 1965. Electrolyte Solutions. Butterworth and Co., Ltd., London.

Tormey, J. M., and J. M. Diamond. 1967. The ultrastructural route of fluid transport in rabbit gall bladder. *J. Gen. Physiol.* **50**:2031.

Wedner, H. J., and J. M. Diamond. 1969. Contributions of unstirred-layer effects to apparent electrokinetic phenomena in the gall-bladder. *J. Membrane Biol.* **1**:92.

Wheeler, H. O. 1963. Transport of electrolytes and water across wall of rabbit gall-bladder. *Am. J. Physiol.* **205**:427.

– E. D. Ross, and K. K. King. 1969. Effect of carbonic anhydrase inhibitors on isolated rabbit gall-bladders. *Am. J. Physiol.* **216**:175.

Whitlock, R. T., and H. O. Wheeler. Coupled transport of solute and water across rabbit gall-bladder epithelium. *J. Clin. Invest.* **43**:2249.

– – 1967. Anion transport by isolated rabbit gall-bladder. *Am. J. Physiol.* **213**:1199.

Windhager, E. E., G. Whittembury, D. E. Oken, H. J. Schatzmann, and A. K. Solomon. 1959. Single proximal tubules of the Necturus kidney. III. Dependence of H_2O movement on NaCl concentration. *Am. J. Physiol.* **197**:313.

Wright, E. M., and J. M. Diamond. 1968. Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions. *Biochim. Biophys. Acta* **163**:57.