Articles

Apical Membrane of the Gastric Parietal Cell: Water, Proton, and Nonelectrolyte Permeabilities†

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ABSTRACT: Gastric parietal cell apical membranes must protect the cell from the extremely low pH and wide variations in osmolality of the gastric juice. To characterize the permeability properties of gastric apical membranes, we have measured passive permeabilities to water, protons, NH_3, and small nonelectrolytes of membrane vesicles derived from parietal cells of fasted animals and fed animals. Both preparations are known to be highly enriched in H^+/K^+-ATPase, the enzyme responsible for acidifying the gastric contents. The preparations behaved as single populations, and their permeability properties were similar in all respects, permitting pooling of the results. This similarity suggests that insertion of tubulovesicles into the apical membrane does not change the behavior of the lipid bilayer. Osmotic water permeability (P_f) averaged (mean ± SD) $(2.8 \pm 0.3) \times 10^{-4}$ cm/s, a value 10-fold lower than that obtained in lecithin large unilamellar vesicles (LUV) and similar to that obtained in other water-tight epithelia. Similarly, ammonia permeability (P_{NH_3}) was low [$(4.4 \pm 2.3) \times 10^{-3}$ cm/s] and 10 times below that of lecithin LUV. By contrast, proton permeability (P_{H^+}) was surprisingly high (0.030 ± 0.011 cm/s) and similar to that of lecithin LUV. These results suggest that the pathway for proton permeation differs from that of water and NH_3. Nonelectrolyte permeabilities were strikingly similar to those obtained in another water-tight epithelium, the toad urinary bladder. Moreover, these permeabilities followed Overton's rule in that permeability varied in accordance with the oil–water partition coefficient. We conclude that the gastric apical membrane, like that of several renal epithelia, is relatively water-tight and exhibits low permeabilities to small nonelectrolytes. These properties are likely to be essential to the ability of this membrane to perform its barrier function.

The gastric juice has a low pH and a widely varying osmolality, yet the epithelial cells lining the stomach must maintain a relatively constant intracellular osmolality and pH. The stomach's ability to maintain a proton concentration gradient of greater than 1 000 000-fold has been termed the gastric mucosal barrier (Hirst, 1990). The physiological gastric mucosal barrier includes several anatomical barriers such as the mucus–bicarbonate layer, the apical epithelial membrane lipid bilayer and tight junctions, intracellular buffering and pH regulation, and mucosal blood flow (Wilkes et al., 1989; Allen et al., 1984; Hirst, 1990). Controversy surrounds the role mucus plays; however, it is unlikely to provide a significant barrier to the diffusion of H^+ since the mucus layer layer exhibits a limited buffer capacity (Hirst, 1990). Also, since H^+ is secreted from the parietal cell beneath the mucosal layer, protons must pass from the parietal cell surface through the mucus to reach the lumen of the stomach. Evidence for the significance of the apical cell membrane in the gastric permeability barrier comes from studies with barrier-breaking agents such as ethanol (Davenport, 1967; Bailey et al., 1987) and aspirin (Davenport, 1965a,b). When such agents disrupt the apical membrane, transepithelial permeabilities to Na^+ and H^+ increase dramatically. In addition, other barrier-breaking agents, such as detergents, bile acids, and phospholipases, have been shown to damage the apical membrane first, and later the parietal cell (Pipkin et al., 1984; Hirst, 1990).

Since the osmolality ingested can range from zero (free water) to several thousand milliosmoles per kilogram, gastric parietal cells must be able to withstand abrupt changes in osmolality. In addition, during gastric acid secretion, the apical surface area of the parietal cell increases enormously to accommodate the large numbers of H^+/K^+-ATPase-containing vesicles which fuse with the apical membrane during secretion (Forte & Soll, 1990; Black et al., 1980). These considerations suggest that the gastric apical membrane must maintain a low permeability to water, so that the gastric epithelial cell is afforded some protection from abrupt changes in cell volume. Other sites at which apical membranes are water-tight include the mammalian kidney and anuran bladder, where apical membranes of cells of the thick ascending limb, collecting duct, and toad bladder granular cells have been shown to maintain strikingly low osmotic water permeabilities (P_f)[1] in the range of 1×10^{-4} cm/s (Finkelstein, 1986; Hebert & Andreoli, 1982; Harris et al., 1991; Kikeri

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et al., 1989). Although apical membranes of toad urinary bladder granular cells have been obtained and their permeabilities characterized (Grossman et al., 1992), low yield has complicated the analysis of their composition and structure.

During fasting, parietal cells contain numerous tubulovesicles, which contain H^+/K^+-ATPase; these vesicles can be isolated from the fasting pig and are highly enriched for the enzyme (Ljungstrom et al., 1984). Within minutes of stimulation by feeding or secretagogues, tubulovesicles fuse with the apical membrane, making it tortuous and highly redundant (Black et al., 1980; Forte & Soll, 1990). Vesicles isolated from pigs with free access to food may contain both subapical vesicles that have not yet fused with the apical membrane and apical membrane vesicles; these preparations are also highly purified for the H^+/K^+-ATPase and have been used to characterize this enzyme (Saccomani et al., 1977; Rabon et al., 1980; Sachs et al., 1990). Interestingly, the Cl^- permeability of apical membrane vesicles in rabbit appears to be much higher than that of subapical vesicles (Wolosin & Forte, 1984; Forte & Soll, 1990). Because the subapical vesicles fuse so promptly with the apical membrane, it appears likely that the permeability properties of their lipid bilayers (i.e., permeabilities to water, protons, and small nonelectrolytes) resemble closely those of the gastric apical membrane. However, this issue has never been explored.

The present studies were designed to determine water, proton, and small nonelectrolyte permeabilities of gastric apical membrane vesicles. By using preparations from both fasted pigs and animals with free access to food, possible differences in passive permeabilities were determined. The results demonstrate that the apical membrane of the gastric mucosa is a relatively water-tight membrane with exceptionally low permeabilities to small nonelectrolytes. The availability of methods to purify these membranes in large quantities and with preservation of their bilayer structure should enhance our understanding of how the lipid composition and bilayer structure of biological membranes result in low water and nonelectrolyte permeabilities.

MATERIALS AND METHODS

Materials. Carboxyfluorescein (CF) was obtained from Molecular Probes, Junction City, OR. Anti-fluorescein antibody was prepared as described (Harris et al., 1990b). *n*-Butyramide was obtained from Fluka Chemical (Ronkonoma, NY). Glycerol and monoethanolamine were obtained from Baker Chemical (Phillipsburg, NJ). Urea was obtained from Bio-Rad Laboratories (Richmond, CA). Propylene glycol and sucrose were obtained from Fisher Scientific (Pittsburgh, PA). Ficoll 400 was obtained from Pharmacia LKB (Uppsala, Sweden). Grade I egg yolk lecithin was obtained from Lipid Products (South Nutfield, U.K.). All other chemicals were obtained from Sigma Chemical (St. Louis, MO).

Solutions. Sucrose buffer contained 200 mM sucrose, 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), 10 mM Tris, and 0.1 EDTA (pH 7.5, 275 mOsm/kg). KCl buffer consisted of 200 mM sucrose, 30 mM KCl, 10 mM HEPES–Tris, and 0.1 mM EDTA (pH 7.5, 275 mOsm/kg).

Vesicle Preparations. Mucosal cells were scraped from the fundi of stomachs of fasted pigs or fed pigs obtained from a local meat packing facility. Subapical vesicles and apical membrane vesicles from pigs with free access to food were prepared by differential and density gradient centrifugation as described (Rabon et al., 1980, 1988; Saccomani et al., 1977). Tubulovesicle-derived membranes from fasted pigs were purified as described (Ljungstrom et al., 1984). Briefly, stomachs were excised and placed in saline, and the interior was washed twice with saturated NaCl in 2–3 min. After the stomach was opened along the greater curvature, the antrum was removed and discarded. The body of the stomach was scraped wherever there were rugae. The scrapings were homogenized in 0.25 M sucrose with seven strokes in a Teflon–glass homogenizer (Glas-col Model K43 stirrer at 1000 rpm, Glas-col Co., Terre Haute, IN; 30-mL glass homogenization tube with fitted Teflon pestle, Fisher Scientific) in 5–6 batches. The homogenate was centrifuged at 20000*g* for 40 min. The pellet was discarded, and the supernatant was centrifuged at 75000*g* for 60 min. The resulting pellet was homogenized in 8.6 mL of 0.25 M sucrose. Aliquots of 4.3 mL were placed in centrifuge tubes on top of step gradients consisting of (from the bottom) 7.14 mL of 37% sucrose and 12.9 mL of 7.5% Ficoll. The tubes were centrifuged at 75000*g* for 1 h. Fractions collected from between the two layers were diluted with 0.25 M sucrose and repelleted for 15 min at 75000*g*. The pellet was suspended in sucrose buffer containing 1.3% glycerol to maintain vesicle integrity and activity of the H^+/K^+-ATPase (Rabon et al., 1980, 1988; Ljungstrom et al., 1984). Aliquots were stored at −80 °C until use.

Preparation of Large Unilamellar Vesicles (LUV). Egg yolk lecithin (1 mg/mL) was prepared by solubilization in methanol–chloroform, drying to constant weight under reduced pressure, and resuspension in sucrose buffer. Vesicles were extruded through 0.1-μm Nucleopore filters using an HPVE-S high-pressure vesicle extruder (Sciema Technical Services, Ltd., Richmond, British Columbia, Canada; Hope et al., 1985). The liposomes were then used in the same manner as the gastric apical membrane vesicles for determination of the passive permeability properties.

Electron Microscopy. To determine the size and morphology of the vesicles, purified vesicle preparations were fixed with glutaraldehyde. Thin sections were cut, and the diameters of 100 vesicles present were photographed at final magnifications of 28900×–30450×. Vesicle diameters were measured with a micrometer, and surface area-to-volume ratios were determined as described (Grossman et al., 1992; Zeidel et al., 1992c).

Permeability Measurements. Unless otherwise indicated, all measurements were made at room temperature.

Water Permeability (P_f). The vesicles were incubated in 10 mM CF at pH 7.0 and 37 °C for 45 min to load them with the fluorophore. The vesicles were then centrifuged at 75000*g* for 1 h to remove extravesicular CF. The pellet was resuspended in 0.5 mL of sucrose buffer. The vesicles were exposed to an osmotic gradient by rapid mixing of equal volumes in an Applied Photophysics SF.17 MV stopped-flow fluorometer with a measured dead time of 0.7 ms. The excitation wavelength was 490 nm; the emission wavelength was filtered with a 515-nm cutoff filter (Zeidel et al., 1992a,b,c). Vesicles were placed in one drive syringe, and hypertonic solution was placed in the other drive syringe. Upon mixing, the abrupt increase in extravesicular osmolality led to an efflux of water from the vesicles, resulting in an increase of intravesicular CF concentration which self-quenches (Harris et al., 1990a). Extravesicular fluorescein was nearly completely quenched with anti-fluorescein antibody (see Figure

[1] Abbreviations: P_f, osmotic water permeability; P_{H^+}, passive proton permeability; P_{solute}, solute permeability; E_a, activation energy; CF, 5,6-carboxyfluorescein; LUV, large unilamellar vesicle(s).

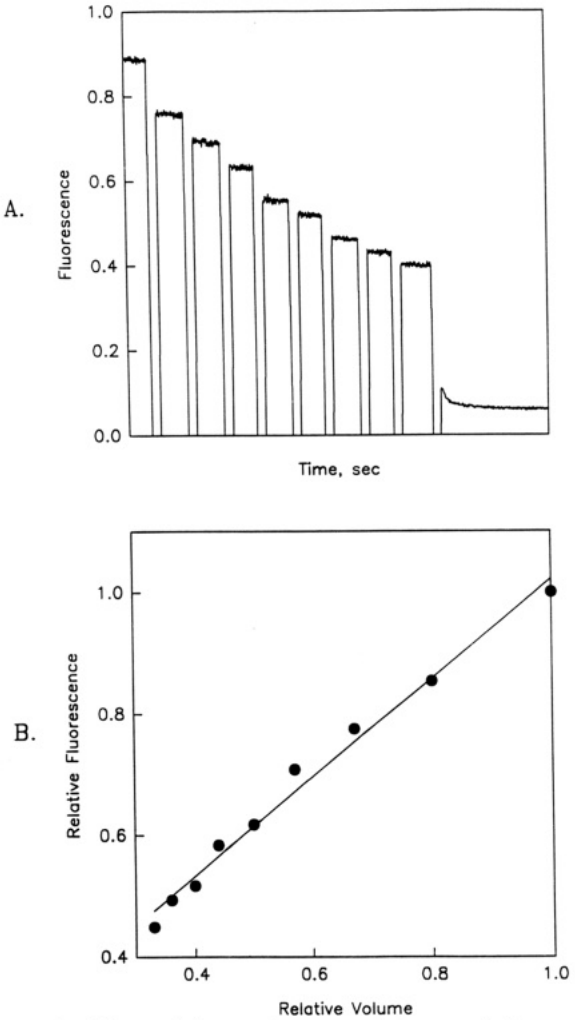

FIGURE 1: Effects of changes in extravesicular osmolality on the fluorescence of entrapped CF. (A) Gastric vesicles with sufficient extravesicular anti-fluorescein antibody to quench extravesicular fluorescence were placed in a cuvette equipped with a magnetic stirrer and subjected to stepwise increments on extravesicular osmolality. As extravesicular osmolality rose, water efflux occurred, reducing intravesicular volume and increasing the self-quenching of entrapped carboxyfluorescein. At the end of the tracing, the microsomes were lysed with Triton X-100, demonstrating that virtually the entire fluoescence signal comes from intravesicular fluorescein. (B) Plot of relative fluorescence vs relative volume using data from panel A. Relative fluorescence was calculated by dividing the fluorescence by the initial fluorescence. Relative volume was calculated from the extravesicular osmolality. These graphs are representative of similar results obtained in five preparations.

1A; Harris et al., 1990a). To enhance the signal-to-noise ratio, 5–10 curves were averaged for each experiment. The relationship between relative volume and relative fluorescence was obtained by measuring the intravesicular osmolality using an SLM-Aminco 500C spectrofluorometer (excitation 490 nm, emission 520 nm). Sucrose was added to the extravesicular solution incrementally, resulting in a stepwise quenching of entrapped CF (Figure 1a). Over the range of osmolalities used, relative fluorescence and relative volume (calculated from extravesicular osmolality) were linearly correlated (Figure 1B, $R = 0.978$). Moreover, plotting of the expected relative volume (calculated from the extravesicular osmolality) and the measured relative volume (obtained from the relative fluorescence and the quench curve for CF in aqueous solution) revealed that the vesicles behaved as perfect osmometers over the range of osmolalities employed (not shown).

Fluorescence data from the stopped-flow device were fitted to single-exponential curves using the software accompanying

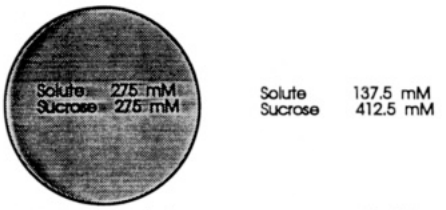

FIGURE 2: Method used for measurements of efflux of small nonelectrolytes from vesicles. Solute concentrations shown inside and outside the vesicles are those present at the instant of mixing in the stopped-flow device.

the Applied Photophysics instrument on a RISC-based Archimedes computer. The software utilizes a nonlinear regression (Marquardt) algorithm calculated from the time course using the "Curfit" routine (Bevington, 1969; Zeidel et al., 1992a). P_f was calculated from the time course of relative fluorescence by comparing single-exponential time constants fitted to simulated curves in which P_f was varied. Simulated curves were calculated using a commercially available software package (MathCad) from the osmotic permeability equation and the relation between relative fluorescence and relative volume:

$$d(V_t)/dt = (P_f)(SAV)(MVW)(C_{in}/V_t - C_{out}) \quad (1)$$

where V_t is the relative volume of the vesicles at time t (i.e., the absolute volume divided by the initial volume), P_f is the osmotic water permeability, SAV is the vesicle surface area-to-volume ratio, MVW is the molar volume of water (18 cm^3/mol), and C_{in} and C_{out} are the initial concentrations of total solute inside and outside the vesicle, respectively (Illsley & Verkman, 1986; Zeidel et al., 1992a–c; Grossman et al., 1992). Since the volume within the vesicles was small compared with the volume outside, it was assumed that C_{out} remained constant throughout the experiment. Parameters from the exponential fit (amplitude and end point) were used to relate relative fluorescence to relative volume using the boundary assumptions that relative fluorescence and volume are 1.0 at time zero and that relative volume reaches a known value (if at time zero the osmolality outside is double that inside, the relative volume reaches 0.5) at the end of the experiment. Single-exponential fits of fluorescence data have been used to determine P_f in a number of vesicle preparations (Illsley & Verkman, 1986; Chen et al., 1988; Meyer & Verkman, 1986; Harris et al., 1990a; Zeidel et al., 1992a), because curves derived from eq 1 do not deviate significantly from single exponentials. In addition, it can be shown mathematically that at earlier time points eq 1 approximates a first-order process (Harris et al., 1990a; Zeidel et al., 1992a).

Small Nonelectrolyte Permeabilities. Permeability of small nonelectrolytes was determined by monitoring relative fluorescence during the efflux of permeant solute. In previous studies (Sha'afi et al., 1971), extravesicular osmolality has been abruptly increased by exposure of the vesicles to increased levels of a permeant extravesicular solute. Such conditions cause an initial rapid efflux of water followed by a slower uptake as the permeant solute enters the vesicles. Since the time courses are biphasic, the calculations of solute permeabilities are difficult and require a number of assumptions and approximations. As shown in Figure 2, for the present measurements the levels of permeant (e.g., urea) and impermeant (sucrose) solutes were higher and lower, respectively, within the vesicle than outside. As the permeant solute effluxes down its concentration gradient from inside to outside, water effluxes, leading to increased CF self-quenching. Because there is initially no osmotic gradient, there is no water flux

except that driven by the efflux of permeant solute. Therefore, the curve is monophasic, and determination of solute permeability is direct and relatively simple (Grossman et al., 1992; Zeidel et al., 1992b).

Vesicles were loaded with appropriate solutes by a 30-min incubation at room temperature. Initial conditions as the two solutions were mixed were 275 mOsm permeant solute and 275 mOsm impermeant solute inside the vesicle and 137.5 mOsm permeant solute and 412.5 mOsm impermeant solute outside the vesicle (see Figure 2). The general formula defining solute flux across a membrane is

$$J_z = dz/dt = (P_z)(SA)(\Delta C)$$

where J_z is the flux of the permeant solute z, P_z is the permeability of the permeant solute z, SA is the surface area of the vesicle, and ΔC is the difference in concentration of the permeant solute between the inside and outside of the vesicle. If

$$V_{rel} = V(t)/V_0$$

where V_0 is the initial volume of the vesicle and V_{rel} and $V(t)$ are the relative and absolute volumes, respectively, at time t, then for our conditions

$$dz/dt = -550(V_0)(dV_{rel}/dt)$$

$$\Delta C = 962.5 - 825/V_{rel}$$

and, therefore

$$dV_{rel}/dt = P_z(SA/V_0)(1/550)(825/V_{rel} - 962.5)$$

By use of parameters from the single-exponential curve fit to the data and the relationship between relative volume and relative fluorescence, P_z was solved using MathCAD (Grossman et al., 1992; Zeidel et al., 1992b).

Proton Permeability. Since the fluorescence of CF varies with pH, the intravesicular pH was monitored by loading vesicles with 0.1 mM CF. As shown in Figure 3A, stepwise reductions in the extravesicular pH led to reductions in the intravesicular pH and in the fluorescence of entrapped CF. Over the pH range used (6.5–7.5), the relative fluorescence was proportional to the relative pH (Figure 3B, $R = 0.984$). P_{H^+} was determined by mixing vesicles equilibrated in KCl buffer including 1.7 μM valinomycin (Wolosin & Forte, 1984) with sucrose buffer containing enough HCl to lower the final extravesicular pH from 7.5 to 6.8. Results were fitted to single exponentials, and the resulting time constant was converted to permeability using the equation:

$$J_{H^+} = P_{H^+}(\Delta C)SA$$

where J_{H^+} is the flux rate of protons, P_{H^+} is the permeability coefficient of protons, ΔC is the concentration gradient for protons across the vesicle membrane at the start of the experiment, and SA is the surface area of the vesicle (Harris et al., 1990b). J_{H^+} was calculated by multiplying $1/\tau$ (where τ is the time constant of the single-exponential curve describing the change in fluorescence as a function of time) by the amount of buffer in an individual vesicle. Buffer capacity was calculated by making 10 mM step additions of acetate and calculating the change in pH from the standard curve (Roos & Boron, 1981). Using the Henderson–Hasselbalch equation, the amount of acetic acid which must have entered the vesicles and dissociated to reduce the intravesicular pH by the amount measured was calculated and the buffer capacity expressed in millimolar per pH unit.

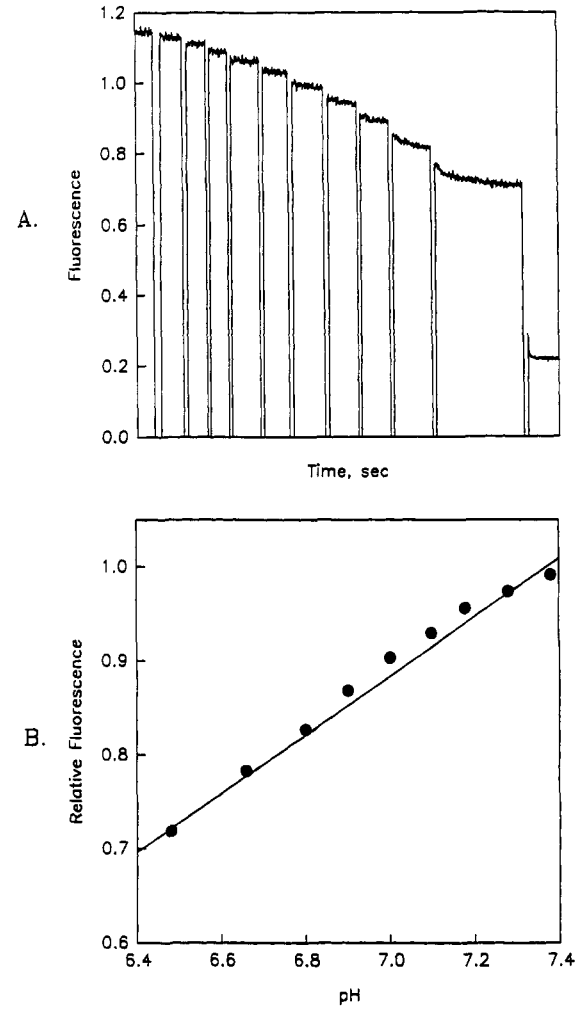

FIGURE 3: Effects of changes in extravesicular pH on quenching of entrapped CF. (A) Vesicles were placed in a cuvette equipped with a magnetic stirrer and subjected to stepwise decrements in extravesicular pH. As extravesicular pH fell, the resulting fall in intravesicular pH reduced the fluorescence of entrapped CF. At the end of the experiment, vesicles were lysed with Triton X-100 as in Figure 1. (B) Relative fluorescence plotted against intravesicular pH; data from panel A.

NH_3 Permeability. P_{NH_3} was determined by rapid mixing of the vesicles at pH 6.8 with ammonium chloride at a final concentration 5 mM. Under these conditions, the small amount of NH_3 present in the ammonium chloride rapidly permeates the membrane. Upon entering the vesicle, the NH_3 titrates a proton, forming NH_4^+ and raising the intravesicular pH (Roos & Boron, 1981). The final intracellular pH was determined from the linear correlation between the relative fluorescence and pH (Figure 3B). Using the rate of change in intravesicular pH and the calculated buffer capacity, P_{NH_3} was calculated.

Statistical Analysis. Values are expressed as means ± SD. Statistical comparisons were made by the Student's t test, paired or unpaired as appropriate, and differences were considered statistically significant when $P < 0.05$.

RESULTS

Vesicle Characterization. Electron micrographs of gastric vesicles from both fed and fasted pig preparations are shown in Figure 4. The average radius of the vesicles from fasted pigs was determined to be $(5.9 \pm 0.9) \times 10^{-6}$ cm (mean ± SE). The vesicles prepared from fed pigs had an average radius of $(6.0 \pm 0.8) \times 10^{-6}$ cm. Both preparations were

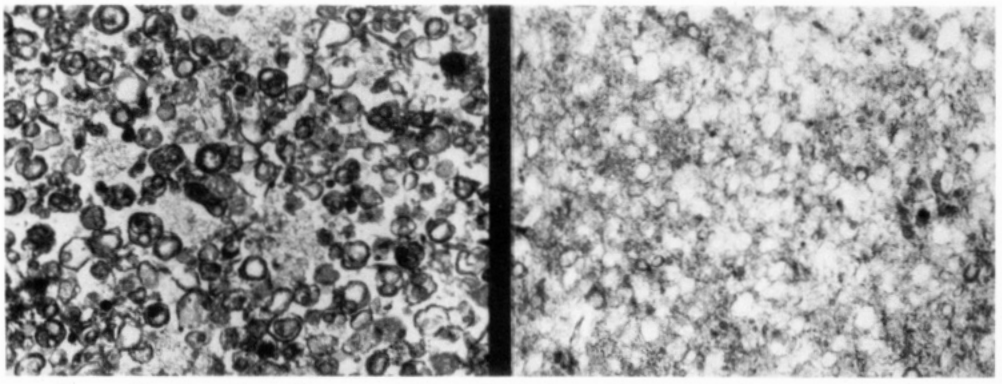

FIGURE 4: Electron micrographs of gastric vesicles prepared from fed (left panel) and fasted (right panel) pigs. The magnification of (A) is 19975×; the magnification of (B) is 19074×.

composed nearly exclusively of vesicular structures, with no mitochondria visible on multiple micrographs. The preparation from pigs with free access to food contained numerous multilamellar vesicles, which characterize preparations of apical membrane from stimulated, actively secreting rabbit stomachs (Forte & Soll, 1990; Black et al., 1980; Wolosin & Forte, 1981). The abundance of multilamellar vesicles may result from the tortuosity of the apical membrane and its fused vesicles during gastric secretion. By contrast, the preparations from fasted pigs consisted almost exclusively of unilamellar vesicles.

The preparation from pigs with free access to food has previously been extensively characterized (Saccomani et al., 1977; Rabon et al., 1988). It exhibits a 95% latent K^+-stimulated ATPase activity of approximately 160 μmol mg^{-1} h^{-1}. Basal Mg^{2+}-dependent ATPase activity is less than 5% of the K^+-stimulated ATPase activity. The phosphorylation capacity is between 1100 and 1300 pmol mg^{-1} h^{-1}. The preparation from fasted pigs has been shown to exhibit an ATPase activity of 48 μmol mg^{-1} h^{-1} (Ljungstrom et al., 1984). SDS–PAGE of each preparation reveals a very prominent band at 100 000 Da, corresponding to the H^+/K^+-ATPase (Saccomani et al., 1977; Ljungstrom et al., 1984). Thus, each preparation is highly enriched in vesicles containing H^+/K^+-ATPase.

Water Permeability. In stopped-flow measurements (Figure 5), the final fluorescence was stable, indicating that sucrose permeation of the vesicles was negligible over the time scale used, and that a steady-state condition had been attained. The data were well fitted with first-order exponential curves, indicating that the process involves a uniform population of vesicles. As shown in Figure 5, measurement of water fluxes under conditions of varying osmotic gradients gave P_f values of 2.2×10^{-4}, 2.4×10^{-4}, and 2.3×10^{-4} cm/s; this independence of the P_f from the osmotic gradient employed demonstrates that the measurements are indeed detecting the flux of water across the membrane. The permeability coefficients of the preparations from fed or fasted pigs were the same, $(2.7 \pm 0.3) \times 10^{-4}$ ($n = 3$) and $(3.0 \pm 0.1) \times 10^{-4}$ cm/s ($n = 2$), respectively; therefore, all were combined, yielding a P_f of $(2.8 \pm 0.3) \times 10^{-4}$ cm/s ($n = 5$). This value was 10-fold slower than that obtained for the control phosphatidylcholine liposomes with a P_f of $(2.6 \pm 0.5) \times 10^{-3}$ cm/s (A. Albalak, J. Donovan, and M. L. Zeidel, unpublished observations).

The activation energy (E_A) of osmotic water flow through the membrane was determined by measuring the rate constant for water flux at different temperatures ranging from 10 to 45 °C. From the Arrhenius plot of ln K vs $1000/T$, the E_A calculated from the slope of the line was found to be 15.1 ± 0.3 kcal mol^{-1} K^{-1} ($n = 4$, Figure 6A, $R = 0.977$). Figure 6B,C shows the effects of $HgCl_2$ on water flow at 37 °C. Water flow at 37 °C averaged $(7.3 \pm 0.7) \times 10^{-4}$ cm/s. With the addition of $HgCl_2$, this value averaged $(7.9 \pm 1.4) \times 10^{-4}$ cm/s. Since water channels display high P_f, low E_A, and sensitivity to mercurials, it is unlikely that water flow through

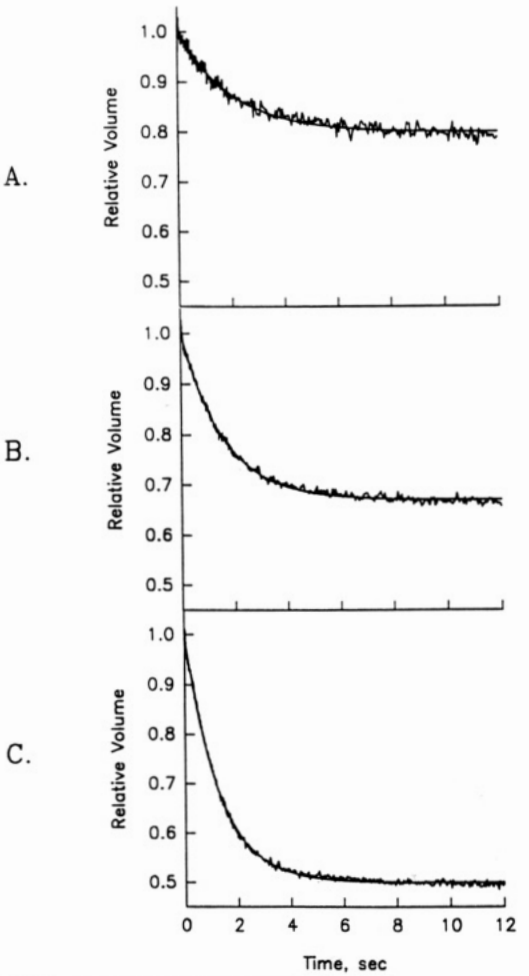

FIGURE 5: Water flux in gastric vesicles. For each panel, extravesicular osmolality was abruptly increased, and as water effluxed from the vesicle and vesicle volume diminished, the reduction in CF fluorescence was monitored fluorometrically. Values of relative fluorescence were converted to relative volume, and the latter were plotted as a function of time. Time courses of volume change are shown with fitted single exponentials. Measurements were performed at room temperature. (A) Extravesicular osmolality was abruptly increased from 275 to 344 mOsm/kg. (B) Extravesicular osmolality was increased from 275 to 412.5 mOsm/kg. (C) Extravesicular osmolality was increased from 275 to 550 mOsm/kg. Results are representative of similar determinations performed on two preparations.

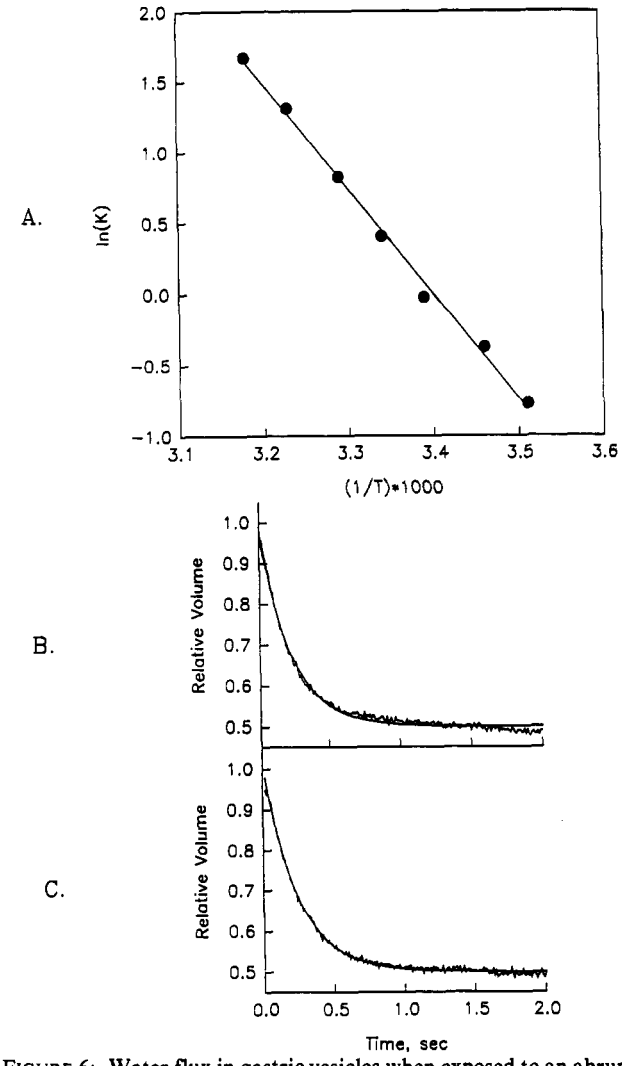

FIGURE 6: Water flux in gastric vesicles when exposed to an abrupt doubling in osmolality. (A) Activation energy (E_A) of osmotic water flow. The ordinate is the natural log of the rate constant for a single-exponential fit of relative fluorescence data (ln K), and the abscissa is the inverse of temperature (T) in degrees kelvin multiplied by 1000. Data are representative of similar results in four preparations. (B) Measurement of water flux at 37 °C; note the increased rate as compared with Figure 2C, due to the higher temperature. (C) Measurement of water permeability at 37 °C in the presence of 0.1 mM $HgCl_2$. Data are representative of similar determinations in three preparations.

gastric vesicles occurs via water channels (Finkelstein, 1986; Zeidel et al., 1992a,b). Because the P_f was low and similar to values obtained in water-tight membranes such as the toad bladder apical membrane in the absence of antidiuretic hormone (ADH) stimulation (Grossman et al., 1992; Finkelstein, 1986; Levine et al., 1984), we examined other permeabilities to compare with the toad bladder apical membrane.

Proton Permeability. The buffer capacity averaged 26.9 ± 7.9 mM/pH unit (n = 4). Similarly, in previous studies using a different method, the buffer capacity ranged between 20 and 90 mM/pH unit (Wilkes et al., 1989). To determine the rate of H^+ permeation, the extravesicular pH was abruptly reduced from pH 7.5 to 6.8 (see Figure 7). Proton permeation occurred at an average rate of 0.275 s^{-1}, yielding a τ of 3.64 s and an average P_{H^+} of 0.030 ± 0.011 cm/s (n = 7). Measurements made on both the fasted and fed pig preparations yielded the same rates and P_{H^+} values. The reverse experiment was run going from pH 6.8 to 7.5, and an inverse curve of similar time course was observed (data not shown). Gramicidin (15 μM) increased the rate by 50-fold going from

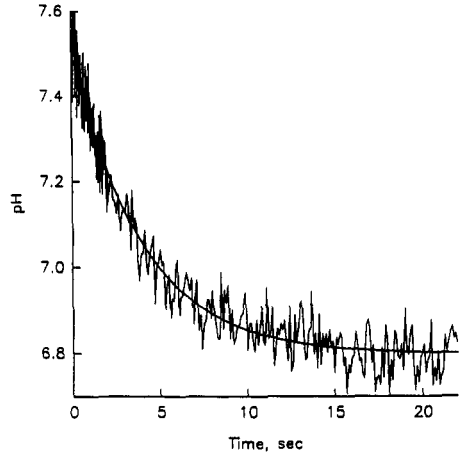

FIGURE 7: Stopped-flow measurement of the rate of change of intravesicular pH in gastric microsomal vesicles. Vesicles were exposed to an abrupt drop in pH from 7.5 to 6.8, and the resulting decline in CF fluorescence was monitored. Shown are data with a fitted single-exponential curve. Results are representative of similar results on seven different preparations.

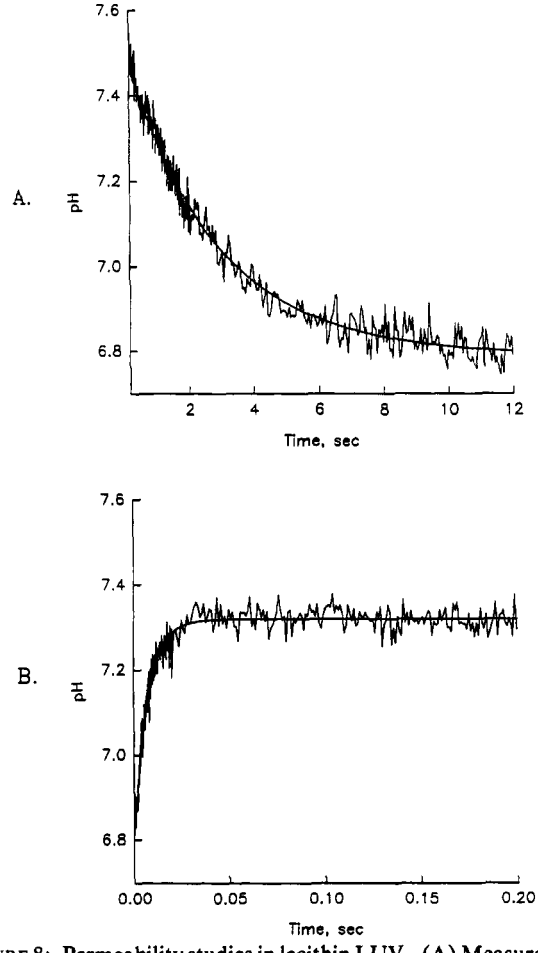

FIGURE 8: Permeability studies in lecithin LUV. (A) Measurement of H^+ permeability by an abrupt drop in pH from 7.5 to 6.8. Shown are data and a fitted single-exponential time course. Results are representative of similar determinations on three preparations. (B) Measurement of NH_3 permeability by abrupt mixing to a final extravesicular NH_4Cl concentration of 10 mM at pH 6.8. Shown are data and a fitted single-exponential time course. Results are representative of similar measurements on three different preparations.

pH 7.5 to 6.8 with the total excursion remaining the same. This result demonstrates that the rate-limiting process in these measurements was the flux of protons across the membrane and confirms the adequacy of the voltage clamp provided by

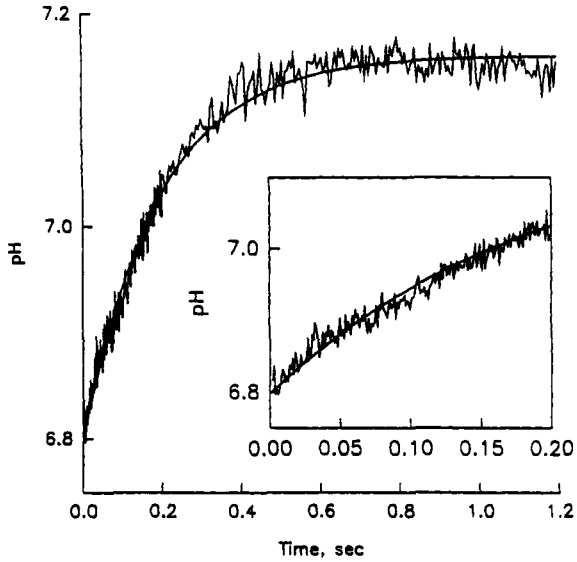

FIGURE 9: NH_3 flux in gastric vesicles. Vesicles in buffer of pH 6.8 were abruptly exposed to 5 mM extravesicular NH_4Cl at pH 6.8, and the increase in fluorescence of entrapped CF was monitored as the intravesicular pH rose. Shown are data and a fitted single-exponential time course. The intravesicular pH (ordinate) was calculated from the standard curve relating intravesicular pH to fluorescence (see Figure 3). Results are representative of similar experiments performed on three different preparations. Inset: Enlargement of early time course.

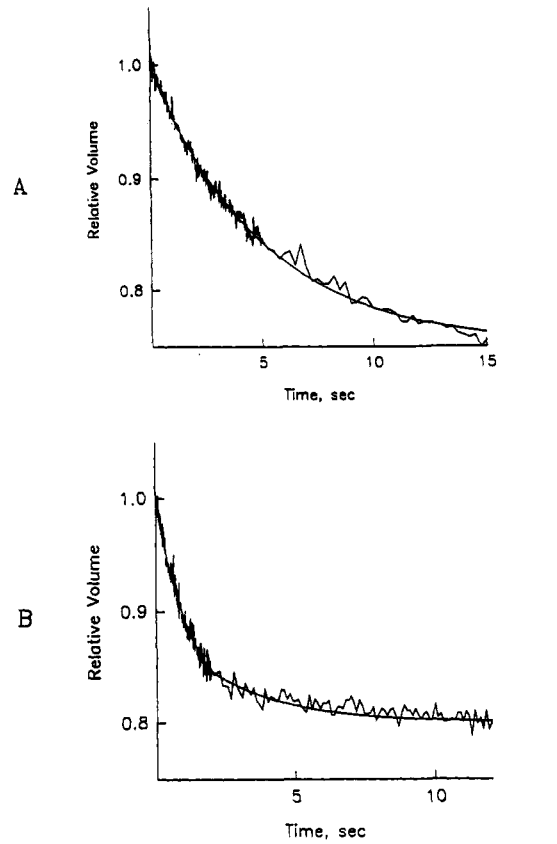

FIGURE 10: Small nonelectrolyte permeabilities of gastric vesicles. (A) Measurement of urea permeability. Shown are data and a fitted single-exponential time course. Results are representative of similar determinations performed on four different preparations. (B) Measurement of acetamide permeability. Shown are data and a fitted single-exponential time course. Results are representative of similar determinations performed on four different preparations.

the valinomycin and K^+. To compare this permeability to that of lecithin LUV, similar measurements were performed in these model membranes (see Figure 8A). The proton permeability of gastric vesicles, 0.030 ± 0.011 cm/s, is comparable to that obtained in the lecithin LUV: 0.026 ± 0.001 cm/s (n = 3).

NH_3 Permeability. The gastric vesicles incubated in KCl buffer at pH 6.8 and 1.7 μM valinomycin were rapidly mixed with ammonium chloride (final concentration, 5 mM). The entry of NH_3 into the vesicles titrated free H^+ and raised the pH, leading to an increase in fluorescence (Figure 9). P_{NH^3} averaged (4.4 ± 2.3) × 10^{-3} cm/s (n = 3). This value is 10-fold slower than that for phosphatidylcholine liposomes; the control liposomes were found to have a P_{NH^3} ranging from 0.03 to 0.11 cm/s (Figure 8B).

Permeabilities to Small Nonelectrolytes: Applicability of Overton's Rule. Permeability coefficients of several solutes (acetamide, butyramide, glycerol, monoethanolamine, propylene glycol, and urea) were determined by loading the vesicles with 550 mOsm permeant solute. After being mixed with an equal volume of 550 mOsm sucrose in the stopped-flow fluorometer, the osmolality was balanced, but an outward gradient existed for the permeant solute while an inward gradient existed for the sucrose (see Figure 2). As a result, the permeant solute moved down the gradient and water effluxed as well to maintain osmotic equilibrium, thus causing the vesicles to shrink and the CF to self-quench further (Figure 10). Since the solute permeabilities were the same for preparations from both fed and fasted animals, the values were combined and are summarized in Table I.

Overton's rule states that the permeability of a solute through a membrane is determined in large part by the oil–water partition coefficient for that solute. To determine whether nonelectrolyte permeation through gastric vesicles confirms Overton's rule, the permeability data were summarized in Table I and plotted against the oil–water partition coefficients for the solutes in several model solvents in Figure 11. The degree of correlation between permeability and oil–water partition coefficients has been taken to indicate the degree to which hydrophobicity or hydrophobic solvency is a factor in determining permeability (Brindslev & Wright, 1976; Wolosin et al., 1978; Wright & Brindslev, 1976; Wright & Pietras, 1974; Walter & Gutknecht, 1986).

DISCUSSION

Epithelia are designed to separate compartments. The apical membrane of gastric epithelia serves the major barrier function of maintaining striking proton gradients (Hirst, 1990). In addition, the epithelial cells lining the stomach tolerate widely varying osmolalities of food intakes without showing cellular shrinkage or swelling. Thus, the stomach requires a barrier between lumen and cell interior to restrict water and H^+ fluxes.

Intact tissue is not suitable to study the permeability properties of gastric apical membranes because the deep gastric crypts set up large unstirred layers and render surface area difficult to calculate. An alternative to using intact tissues is the use of a membrane preparation. Unilamellar vesicles from fasted pigs and a mixture of unilamellar and multilamellar vesicles from fed pigs were used in these studies. Vesicles prepared from fasting animals represent tubulovesicle membranes prior to insertion into the apical membrane upon stimulation (Ljundstrom et al., 1984). Figure 4 suggests that vesicles prepared from pigs with free access to food frequently contain multilaminar structures, as expected for a mixture of tubulovesicles and apical membranes (Saccomani et al., 1977; Rabon et al., 1980, 1988). Passive permeability properties were examined in both preparations to test whether these

Table I[a]

solute (*n*)	P_x, stomach, cm/s (±SD)	P_x, toad bladder,[b] cm/s (±SD)	$K_{olive\ oil}$	$K_{hexadecane}$	$K_{octanol}$	K_{ether}
acetamide (4)	$(2.3 \pm 0.6) \times 10^{-6}$	$(1.2 \pm 0.1) \times 10^{-6}$	8.3×10^{-3}	2.1×10^{-5}	8.9×10^{-2}	2.5×10^{-3}
butyramide (4)	$(2.6 \pm 1.7) \times 10^{-6}$	$(1.4 \pm 0.1) \times 10^{-6}$	1.7×10^{-2}	3.6×10^{-4}	6.2×10^{-1}	5.8×10^{-2}
glycerol (4)	$(4.9 \pm 2.6) \times 10^{-7}$	$(2.2 \pm 0.3) \times 10^{-7}$	7.0×10^{-5}	2.0×10^{-6}	1.1×10^{-2}	6.6×10^{-4}
ethanolamine (3)	$(1.4 \pm 0.1) \times 10^{-6}$	ND[c]				1.3×10^{-3}
propylene glycol (4)	$(2.0 \pm 0.5) \times 10^{-6}$	ND	1.7×10^{-3}	6.5×10^{-5}	4.4×10^{-2}	1.8×10^{-2}
urea (4)	$(5.5 \pm 1.4) \times 10^{-7}$	$(2.1 \pm 0.2) \times 10^{-7}$	1.5×10^{-4}	2.8×10^{-7}	2.6×10^{-2}	4.7×10^{-4}

[a] P_x, permeability of solute x. Partition coefficients (K) are taken from the following references: Colander, 1949, 1954; Hansch & Anderson, 1967; Leo et al., 1971; Brindslev & Wright, 1976; Finkelstein, 1976; Walter & Gutknecht, 1986; Wolosin et al., 1978. [b] Data from Grossman et al. (1992). [c] Not determined.

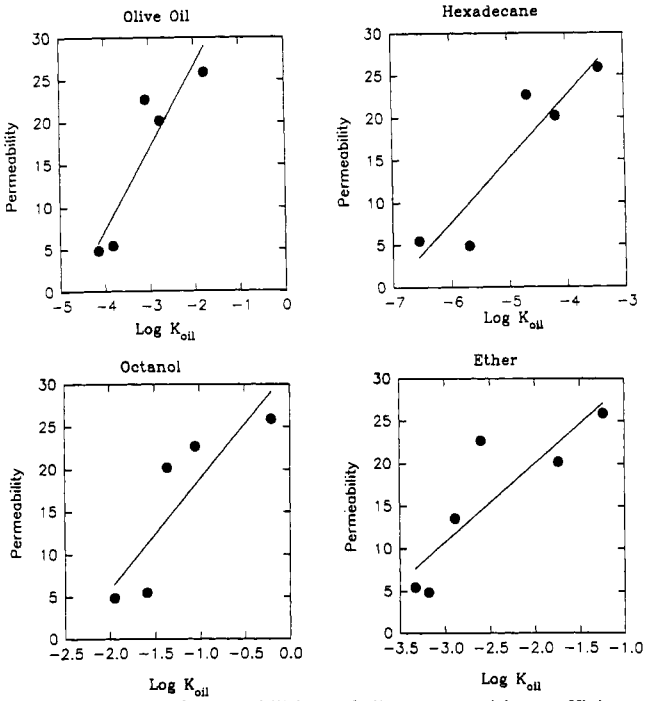

FIGURE 11: Plots of permeabilities and oil–water partition coefficients (K_{oil}) for olive oil ($R = 0.913$), hexadecane ($R = 0.925$), octanol ($R = 0.861$), and ether ($R = 0.866$) as indicated by the respective solvent on the abscissa.

permeabilities changed between the fasting and fed states. Electron micrographs revealed frequent multilamellar vesicles present in the preparation from fed pigs, while fasted pigs exhibited relative ultrastructural homogeneity. The fact that they both behaved as uniform populations in measurements of multiple permeabilities indicates that the permeabilities obtained in these studies are applicable to the entire population of membranes in these preparations.

Previous estimates of the water permeability of gastric apical vesicles from fed pigs using light scattering as a measure of volume yielded an approximate value of 10^{-4} cm/s (Rabon et al., 1980). This value agrees well with the present studies. We also found the same P_f for preparations made from both fed and fasted pigs. These data, along with the measurements of other permeabilities, suggest that the gastric apical membrane and the subapical vesicles which fuse with it consist of a single population whose passive permeability properties do not change upon stimulation.

By contrast, Wolosin and Forte (1984) reported that the density of membrane-containing H^+/K^+-ATPase increased after the stomach was stimulated. In addition, Cl^- permeability increased markedly in apical as compared with tubulovesicles (Wolosin & Forte, 1981, 1984; Forte & Soll, 1990). These studies compared vesicle permeability in rabbit preparations which had been subjected to a maximal secretory stimulus. Thus, the preparation of apical vesicles was highly enriched for multilamellar vesicles and exhibited an increased density relative to the unilamellar tubulovesicles. In addition, the increased Cl^- permeability likely reflects activation of a Cl^- pathway without significant effects on the lipid bilayer. Thus, it appears likely that the lipid bilayer obtained in each preparation is similar.

The P_f of $(2.8 \pm 0.3) \times 10^{-4}$ cm/s signifies a low water permeability. This value is similar to values obtained in a known water-tight epithelium, the toad urinary bladder in the absence of antidiuretic hormone (ADH) stimulation (Grossman et al., 1992). In this tissue, the transepithelial P_f is approximately 2×10^{-4} cm/s (Finkelstein, 1986; Levine et al., 1984), and the P_f of apical membrane vesicles isolated from the bladder averaged $(3.9 \pm 0.4) \times 10^{-4}$ cm/s (Grossman et al., 1992). Thus, the gastric membrane preparations from both fasting and fed animals are water-tight. Tight epithelia such as the kidney collecting duct and the toad urinary bladder exhibit low permeabilities to water, protons, NH_3, and small nonelectrolytes (Grossman et al., 1992; Kikeri et al., 1989; Harris et al., 1992; Brindslev & Wright, 1976). We therefore examined the passive permeabilities of the gastric preparation to a variety of solutes.

The proton permeability value measured, 0.030 ± 0.011 cm/s, is very high, even greater than the previous P_{H^+} value of 3.77×10^{-4} cm/s (Wilkes et al., 1989). The previous value was obtained in membrane vesicles from rabbits in which gastric secretion was maximally stimulated. Under these conditions, the vesicles obtained are predominantly multilamellar (Wolosin & Forte, 1981). Such vesicles are likely to give lower permeability values than predominantly unilamellar vesicles. However, both values of P_{H^+} are inconsistent with known and implied properties of the biological membrane in situ. Values for P_{H^+} estimated for intact gastric mucosa are in the range $(0.4–4.5) \times 10^{-5}$ cm/s. To maintain the observed pH gradient, however, a proton permeability of 10^{-7} cm/s appears to be required (Wilkes et al., 1989). These high proton permeabilities, which were observed at or near neutral pH, may differ from those exhibited at a very low pH on the extracytoplasmic side of the membrane. Further studies measuring P_{H^+} in these vesicles with proton gradients similar to those in situ will be needed to address this question. However, recent studies have defined mechanisms, such as viscous fingering, which permit gastric mucin to sequester acidic gastric juice away from the epithelial cells (Bhaskar et al., 1992). This ability of the gastric mucin to protect the apical surface of the parietal cell from acid may reduce the importance of the apical plasma membrane in preventing backflux of acid into the cell.

While the H^+ permeability of gastric microsomes was higher than expected, the NH_3 permeability was found to be low. This low permeability to NH_3 is also shared by the apical membrane of another water-tight epithelium, the thick

ascending limb of Henle. Both P_{NH_3} and P_f of gastric microsomal vesicles were 10 times lower than the values obtained in LUV. In contrast, the LUV P_{H^+} was 0.026 ± 0.001 cm/s compared with 0.030 ± 0.011 cm/s for the gastric microsomal vesicles. These permeabilities suggest that protons pass through the membrane via a different mechanism than water and NH_3 do.

Small nonelectrolytes were found to obey Overton's law. This finding is contrary to an earlier study in the intact toad bladder in which small solutes such as urea were thought to exhibit high permeabilities relative to their oil–water partition coefficients, suggesting passage through aqueous defects in membrane structure (Brindslev & Wright, 1976; Wolosin et al., 1978; Trauble, 1971). However, follow-up studies in artificial membranes and isolated toad bladder apical membranes (Walter & Gutknecht, 1986; Grossman et al., 1992) have shown that small solutes do follow Overton's rule. The toad bladder apical membrane preparation permeability coefficients are similar to those of the gastric microsomal vesicles, differing by a factor of 2. In agreement with Overton's rule, linear correlations were observed between permeability coefficients describing solute fluxes in gastric microsomal vesicles and the solvent–water partition coefficients.

Although water-tight membranes have now been isolated and their permeability properties characterized, how lipid composition and bilayer structure result in these unique permeability properties remains unknown. Studies in artificial membranes revealed that varying the lipid composition led to changes in permeabilities, which corresponded to changes in membrane fluidity (Finkelstein, 1986; Orbach & Finkelstein, 1980). Thus, additions of cholesterol and sphingomyelin to lecithin bilayers resulted in more water-tight membranes (Finkelstein, 1986; Fettiplace & Haydon, 1980). Moreover, permeabilities and fluidity of erythrocyte and brush border membranes can be altered by addition of cholesterol (Deuticke & Ruska, 1976; Storch & Schachter, 1984). However, biological membranes exhibit striking asymmetries in bilayer structure, with the extracytoplasmic membrane leaflet differing from the cytoplasmic leaflet (van Meer, 1990). Indeed, phospholipid flippases, which mediate movement of phospholipids from one leaflet to another, are likely to be important in generating and maintaining these asymmetries in leaflet composition (Zachowski et al., 1986; van Meer, 1990). Studies in cultured epithelial cells have demonstrated that the tight junctions between cells permit lipid molecules of the cytoplasmic leaflet but not the exofacial leaflet to intermingle between the apical and basolateral membrane domains (Simons & Fuller, 1985; van Meer, 1990). Thus, in intact cells, differences in permeabilities and composition of apical and basolateral membrane domains are likely to reside in the exofacial leaflet. At present, there is no information as to the relative importance of lipid composition and bilayer asymmetry in the barrier function of apical membranes.

Gastric microsomes prepared from fasted pigs have three attractive features for future studies to examine these issues: (1) the lipid composition has already been analyzed; (2) the preparation can be made in large quantities, permitting preparation of LUV from lipid extracts of these preparations; and (3) the vesicles are unilamellar and all oriented cytoplasmic side out, permitting analysis of differences in composition of the two membrane leaflets.

In summary, gastric vesicles from fasted and fed animals exhibited similar permeability properties; both preparations exhibited strikingly low permeabilities to water, NH_3, and small nonelectrolytes, while proton permeability was high. The availability of biological membranes of low water permeability will permit exploration of how biological membranes achieve these extraordinary permeability properties.

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REFERENCES

Allen, A., Flemstrom, G., Garner, A., Silen, W., & Turnberg, L. A., Eds. (1984) *Mechanisms of mucosal protection in the upper gastrointestinal tract*, Raven Press, New York.

Bailey, R. E., Levine, R. A., Nandi, J., Schwartzel, E. H., Jr., Beach, D. H., Borer, P. N., & Levy, G. C. (1987) *Am. J. Physiol. 252*, G237–G243.

Bevington, P. R. (1969) *Data reduction and error analysis for the physical sciences*, McGraw-Hill, New York.

Bhaskar, M. R., Garik, P., Turner, B. S., Bradley, J. D., Bansil, R., Stanley, H. E., & LaMont, J. T. (1992) *Nature 360*, 458–461.

Black, J. A., Forte, T. M., & Forte, J. G. (1980) *Anat. Rec. 196*, 163–172.

Brindslev, N., & Wright, E. M. (1976) *J. Membr. Biol. 29*, 265–288.

Collander, R. (1949) *Acta Chem. Scand. 3*, 717–747.

Collander, R. (1954) *Physiol. Plant 7*, 420–445.

Davenport, H. W. (1965a) *Gastroenterology 49*, 189–196.

Davenport, H. W. (1965b) *Gastroenterology 49*, 238–245.

Davenport, H. W. (1967) *Proc. Soc. Exp. Biol. Med. 126*, 657–662.

Deuticke, B., & Ruska, C. (1976) *Biochim. Biophys. Acta 433*, 638–653.

Fettiplace, R., & Haydon, D. A. (1980) *Physiol. Rev. 60*, 510–550.

Finkelstein, A. (1976) *J. Gen. Physiol. 68*, 127–135.

Finkelstein, A. (1986) *Water movement through lipid bilayers, pores and plasma membranes: theory and reality*, Wiley and Sons, New York.

Forte, J. G., & Soll, A. (1990) in *Handbook of Physiology: The gastrointestinal system, gastrointestinal secretion*, Chapter 11, pp 207–228, American Physiological Society, Bethesda, MD.

Grossman, E. B., Harris, H. W., Star, R. A., & Zeidel, M. L. (1992) *Am. J. Physiol. 262*, C1109–C1118.

Hansch, C., & Anderson, S. M. (1967) *J. Org. Chem. 32*, 2583–2586.

Harris, H. W., Jr., Handler, J. S., & Blumenthal, R. (1990a) *Am. J. Physiol. 258*, F237–F243.

Harris, H. W., Kikeri, D., Janoshazi, A., Solomon, A. K., & Zeidel, M. L. (1990b) *Am. J. Physiol. 259*, F366–F371.

Harris, H. W., Strange, K., & Zeidel, M. L. (1991) *J. Clin. Invest. 88*, 1–8.

Hebert, S. C., & Andrioli, T. E. (1982) *Kidney Int. 22*, 526–535.

Hirst, B. H. (1990) in *Handbook of Physiology: The gastrointestinal system, gastrointestinal secretion*, Chapter 15, pp 279–308, American Physiological Society, Bethesda, MD.

Hope, M. J., Bally, M. B., Webb, G., & Cullins, P. R. (1985) *Biochim. Biophys. Acta 812*, 55–65.

Illsley, N. P., & Verkman, A. S. (1986) *J. Membr. Biol. 94*, 267–278.

Kikeri, D., Sun, A., Zeidel, M. L., & Hebert, S. C. (1989) *Nature 339*, 478–480.

Leo, A., Hansch, C., & Elkins, D. (1971) *Chem. Rev. 71*, 525–616.

Levine, S. D., Jacoby, M., & Finkelstein, A. (1984) *J. Gen. Physiol. 83*, 529–541.

Ljungstrom, M., Norberg, L., Olaisson, H., Wernstedt, C., Vega, F. V., Arvidson, G., & Mardh, S. (1984) *Biochim. Biophys. Acta 769*, 209–219.
Orbach, E., & Finkelstein, A. (1980) *J. Gen. Physiol. 75*, 427–436.
Pipkin, G., Price, C. A., & Parsons, M. E. (1984) *Gastroenterology 87*, 1283–1291.
Rabon, E., Tacheguchi, N., & Sachs, G. (1980) *J. Membr. Biol. 53*, 109–117.
Rabon, E. C., Im, W. B., & Sachs, G. (1988) *Methods Enzymol. 157*, 649–654.
Roos, A., & Boron, W. F. (1981) *Physiol. Rev. 61*, 296–432.
Saccomani, G., Stewart, H. B., Shaw, D., Lewin, M., & Sachs, G. (1977) *Biochim. Biophys. Acta 465*, 311–330.
Sachs, G., Kaunitz, J., Mendlein, J., & Wallmark, B. (1990) in *Handbook of Physiology: The gastrointestinal system, gastrointestinal secretion*, Chapter 12, pp 229–253, American Physiological Society, Bethesda, MD.
Sha'afi, R. I., Gary-Bobo, C. M., & Solomon, A. K. (1971) *J. Gen. Physiol. 58*, 238–258.
Storch, J., & Schachter, D. (1984) *Biochemistry 23*, 1165–1170.
Trauble, H. (1971) *J. Membr. Biol. 4*, 193–208.
van Meer, G. (1990) *Annu. Rev. Cell Biol. 5*, 247–275.
Walter, A., & Gutknecht, J. (1986) *J. Membr. Biol. 90*, 207–217.
Wilkes, J. M., Ballard, H. J., Dryden, D. T. F., & Hirst, B. H. (1989) *Am. J. Physiol. 256*, G553–G562.
Wolosin, J. M., & Forte, J. G. (1981) *J. Biol. Chem. 256*, 3149–3152.
Wolosin, J. M., & Forte, J. G. (1984) *Am. J. Physiol. 246*, C537–C545.
Wolosin, J. M., Ginsburg, H., Lieb, W. R., & Stein, W. D. (1978) *J. Gen. Physiol. 71*, 93–100.
Wright, E. M., & Pietras, R. J. (1974) *J. Membr. Biol. 17*, 293–312.
Wright, E. M., & Bindslev, N. (1976) *J. Membr. Biol. 29*, 289–312.
Zachowski, A., Favre, E., Cribier, S., Herve, P., & Devaux, P. F. (1986) *Biochemistry 25*, 2585–2590.
Zeidel, M. L., Albalak, A., Grossman, E. B., & Carruthers, A. (1992a) *Biochemistry 31*, 589–596.
Zeidel, M. L., Ambudkar, S. V., Smith, B. L., & Agre, P. (1992b) *Biochemistry 31*, 7436–7440.
Zeidel, M. L., Hammond, T. G., Botelho, B., & Harris, H. W. (1992c) *Am. J. Physiol. 263*, F62–F76.