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Water and Salt Permeability of Gastric Vesicles

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Summary. Volume-dependent changes in light scatter have been shown to be a linear function of the osmotic gradient imposed upon gastric vesicles purified from hog gastric mucosa. Observation of the light scattered 90° to incident, using the Durrum stop flow system D-110, indicates that the vesicles exposed to hypertonic medium undergo rapid shrinkage due to water loss from the vesicle interior. The rate constant for this water movement is 1.1 ± 0.09 sec^{-1} ($n=10$) and is linearly dependent on temperature between 16 and 36 °C. The activation energy of 13.93 ± 0.60 kcal mole^{-1} ($n=3$), calculated from an Arrhenius plot, is inconsistent with water movement facilitated by a large-pore aqueous channel. A slower reswell phase, dependent on solute entry into the intravesicular space, follows the water-dependent shrink phase. KCl entry, studied because of the intravesicular requirement for active K^+/H^+ transport, exhibits two entry stages. The faster, described by a single exponential imposed upon a constantly sloping background, has a rate constant of $7.75 \pm 0.48 \times 10^{-3}$ sec^{-1} ($n=15$). The slower phase, which typically accounts for 90% of the reswell process, demonstrates a rate constant of $1.94 \pm 0.23 \times 10^{-4}$ sec^{-1} ($n=15$). In the presence of valinomycin or nigericin, two fast rate constants and one slow rate constant of swelling are observed. The rate constant of the faster reswell phase is increased from $7.75 \pm 0.48 \times 10^{-3}$ sec^{-1} ($n=15$) to $15.74 \pm 3.7 \times 10^{-3}$ sec^{-1} ($n=5$) and $17.23 \pm 3.4 \times 10^{-3}$ ($n=3$) by the addition of nigericin (1 μg ml^{-1}) and valinomycin (4.5 μM), respectively. The second part of the faster reswell phase is approximately that seen in the control population. Transport-dependent volume changes of significant magnitude can be demonstrated following the addition of ATP to vesicles equilibrated with 150 mM KCl. The volume change is a function of HCl leak rate and is abolished by ionophores which eliminate the transport-dependent pH gradient. SO_4^{--} substitution, which eliminates the overshoot phenomena observed in KCl medium, also eliminates the shrinkage resulting from ATP addition.

Recently plasma membrane vesicles derived from animal cell membranes have provided a fresh approach to the complicated transport characteristics of the intact cell. This has been true particularly of the intestinal (Hopfer et al., 1973) and renal brush border (Kinne et al., 1975) as examples of Na^+ symport transport processes, and of gastric parietal cell vesicles as an example of an ATP activated process (Sachs et al., 1977). In the latter example it has been determined that the addition of ATP results in H^+ uptake apparently in exchange for intravesicular K^+ (Sachs et al., 1976) by an electroneutral exchange mechanism. By isotopic and other techniques it seems also that in the vesicle transport reactions, the rate-limiting step is KCl entry (Schackmann et al., 1977).

Osmotic shrinkage swelling techniques were pioneered for studies of water and solute transport in red cells (Sha'afi, Gary-Bobo & Solomon, 1971, and Owen & Eyring, 1975), both with continuous flow and stop flow kinetic methods. These are based on the scattering of incident light that increased with decreasing cell volume. The same approach has been applied to studies of mitochondrial transport (Tedeschi & Harris, 1955), brain microsomes (Kamino & Inoue, 1969), liposomes (Bangham, DeGier & Greville, 1967), and, quite recently, to sarcoplasmic reticulum vesicles (Kometani & Kasa, 1978) and in a preliminary fashion to gastric vesicles (Sachs, Rabon & Saccomani, 1979). In this paper we examine in some detail the justification for application of osmotic shrinkage swelling technique to purified gastric vesicles, as well as the pathways available for H_2O and

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cations in the vesicle membrane. This approach provides a method for quantitating these pathways. In addition, it allows the determination of any osmotic changes which might occur during active transport, which may confirm or deny previous hypotheses relating to active transport.

Materials and Methods

Vesicle Preparation

Hog gastric vesicles were obtained by previously published methods (Rabon et al., 1978*a*). Briefly, this involved removal of the surface cells by NaCl flooding, homogenization of the tissue scrapings above the internal muscularis in an isolation solution consisting of 0.25 M sucrose, 2 mM PIPES/Tris, pH 6.1, differential centrifugation to obtain the post $20{,}000 \times g$ microsomal pellet and ficoll-sucrose step gradient centrifugation in a zonal rotor to provide a light membrane fraction that shows high H^+ uptake activity upon ATP addition to KCl equilibrated vesicles.

Transport of H^+

This was monitored by previously detailed methods using pH electrode (Sachs et al., 1976). The vesicles were pre-equilibrated by a 2 h room temperature incubation with 150 mM KCl, 2 mM $MgCl_2$, 1 mM PIPES/Tris pH 6.1, and protein 0.167 mg ml^{-1}. At zero time, 0.8 mM MgATP was added to the vesicle suspension at room temperature and the time course of the change in external pH was recorded. The signal excursion was quantitated by the addition of a known quantity of acid at the end of each experiment.

H_2O Transport

The principle of the technique was to apply an osmotic gradient to vesicles suspended in 0.25 M sucrose. Sucrose equilibration is accomplished during the isolation procedure, which utilizes 0.25 M sucrose in all purification steps and is maintained by vesicle storage in 0.25 M sucrose, 1 mM PIPES/Tris, pH 6.1 at 4 °C during the week of experimentation. In preliminary experiments the rate of shrinkage for a given difference in osmotic pressure was independent of the nature of the solute provided it had low permeability. Accordingly, we will describe only data obtained for osmotic gradients of KCl. The experiments were carried out in a Durrum system 110 stop flow spectrophotometer equipped with a fluorescent cell attachment to permit perpendicular placement of the photomultiplier tube. Ninety-degree light scatter, observed as either a change in transmittance or absorbance at 500 nm, was monitored on a Tektronix storage oscilloscope equipped with a Polaroid camera. Temperature control was via circulation pump coupled to a Lauda temperature regulated water bath. All solutions were deaerated prior to use to minimize air bubble formation, and were subsequently equilibrated in the injection syringes located in the water bath for 10 min before mixing.

Since light scattering was used as a measure of vesicle volume, it was necessary to calibrate the scatter change within the range of osmotic pressures studied. The technique used for the calibration procedure was to mix in a 1:1 ratio, the vesicles (0.075 mg ml^{-1} protein) suspended in 0.25 M sucrose, 2 mM $MgCl_2$, 2 mM PIPES/Tris (pH 7.4), with an identical solution minus protein and containing successive increments of KCl. Manipulations of KCl concentration provided an osmotic gradient within the range of 70 to 1484 mosmol. For temperature-dependent studies, temperature was varied between 16 and 36 °C in order to obtain the activation energy of water flow. Because the reaction chambers are composed of Kel-F, the circulating water bath was maintained at each temperature for 20 min before solutions were loaded into the reservoir syringes, and at temperature equilibrium, a constant gradient of 138 mosmol was used to initiate water movement. The best fit of the data permitted analysis of water movement by a single exponential.

Salt Transport

It has been demonstrated previously (Sha'afi, Gary-Bobo & Solomon, 1971; Owen & Eyring, 1975) and confirmed in these studies that the reswelling phase is a function of solute entry. In this particular case, salt transport, the entry process is slow enough that shrinkage and swelling can be analyzed as separate processes, which is not true of rapidly penetrating solutes.

In the present investigation, a fixed osmotic gradient of 138 mosmol KCl, was applied to the vesicles. In certain experiments, ionophores such as valinomycin (4.5 μM) or nigericin (1 μg ml^{-1}) were added to the reaction mixture. Initially, vesicles (0.075 mg ml^{-1}) were suspended in 0.25 M sucrose, 2 mM $MgCl_2$ and 2 mM PIPES/Tris pH 7.4. The salt gradient was established by pulsing with an equal volume of solution containing 0.25 M sucrose, 150 mM salt, 2 mM $MgCl_2$ and 2 mM PIPES/Tris pH 7.4. The temperature was maintained at 25 °C. The results were recorded on a Radiometer Servorecorder and the rate constant determined for each slope present by a semilog plot of voltage vs. time. A constantly sloping background was subtracted from each voltage measurement, and the rate constant calculated from the subsequent slope of voltage vs. time.

Osmotic Effects of ATP

Osmotically equivalent solutions were mixed in the stop flow spectrophotometer, with the addition of 1.6 mM ATP to the solute syringe. In addition, the vesicle syringe contained vesicles at 0.086 mg ml^{-1} protein pre-equilibrated with 150 mM KCl, 2 mM $MgCl_2$ and with or without 2.0 mM PIPES/Tris buffer, pH 7.4. Control studies showed that without ATP no changes in light scatter were observed. The rate of change of volume was analyzed as a single exponential imposed upon a constantly sloping background in a manner similar to that used in calculating salt transport. The change in volume was estimated by comparison of the ATP-dependent scatter change to the signal excursion of the calibration curves obtained from the osmotic gradient experiments. On occasion 138 mosmol K_2SO_4 replaced KCl in both syringes.

Protein was measured according to Lowry et al., 1951. ATPase activity was measured as previously detailed (Sachs et al., 1976) with Pi release measured by the method of Yoda and Hokin (1970). All chemicals were of reagent grade. ATP was obtained from Sigma Chemical Company and was vanadate free.

Results

Calibration of Volume Changes

Since the amount of scattered light is a function of the vesicle size, volume changes in the vesicle population can be estimated provided the increase in light scattering is a linear function of vesicle volume. In

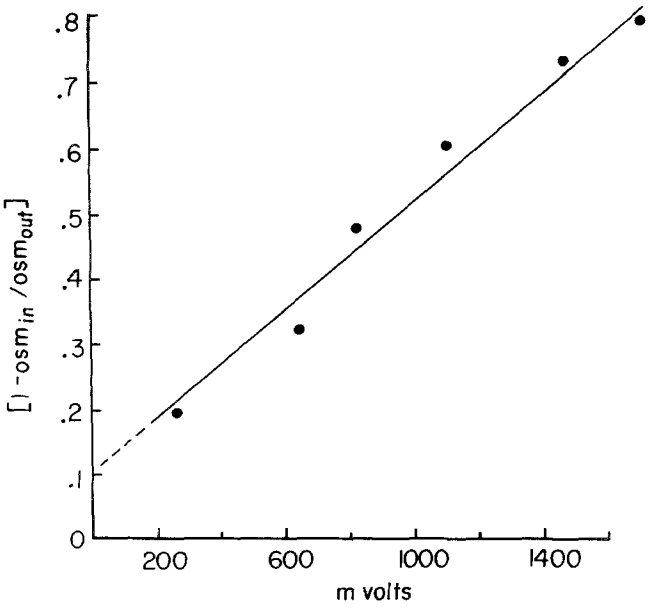

Fig. 1. Volume change of gastric vesicles as a function of KCl concentration gradient. See Materials and Methods for details. Vesicles suspended in 294 mosmol sucrose medium were pulsed with solutions made increasingly hypertonic by KCl addition. The fractional volume change was calculated as ($1-osmolarity_{in}/osmolarity_{out}$) and is displayed as a function of increased scattered light (mV)

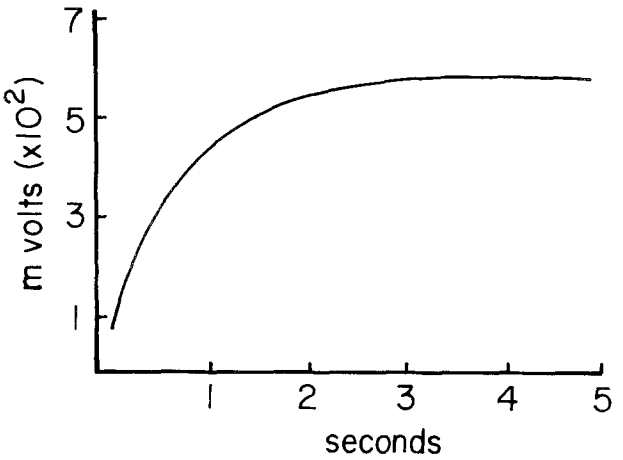

Fig. 2. Rate of vesicle shrinkage due to H_2O efflux following application of a 138 mosm hypertonic KCl solution. See Materials and Methods for details

this determination of osmotically sensitive intravesicular space, the external medium osmolarity was varied by successive increments of KCl to the medium otherwise iso-osmotic with the equilibrated intravesicular space. The hypertonically induced shrinkage at 25 °C was then recorded as the ensuing 90° transmittance change. The total magnitude of this 90° transmittance change was then plotted as a function of the fractional volume change (i.e., l-osmolarity$_{in}$/osmolarity$_{out}$). This plot is shown in Fig. 1, over a range

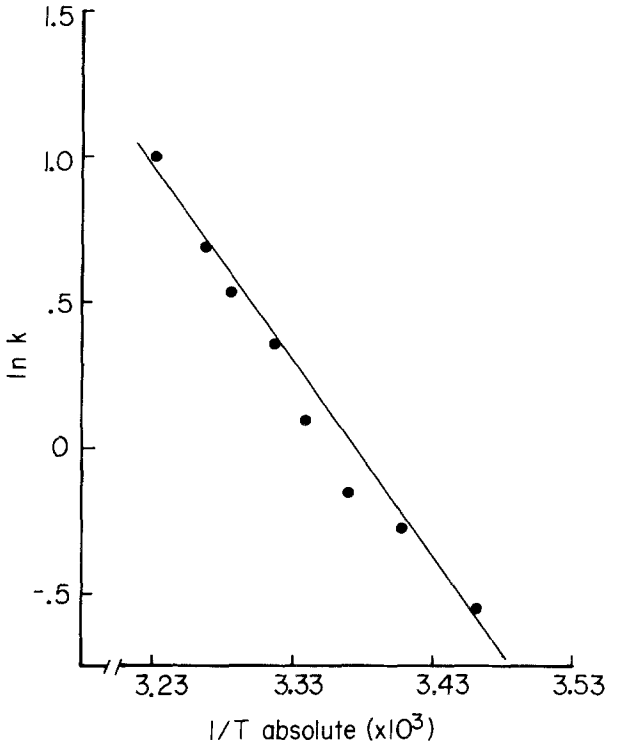

Fig. 3. Arrhenius plot measuring activation energy for H_2O transport in gastric vesicles exposed to hypertonic KCl. See Materials and Methods for details. Activation energy is calculated as 1.987 cal mol^{-1} × slope

of external KCl concentrations from 37.5 mM to 750 mM. Within this osmotic range, the relationship between the degree of vesicle shrinkage (an increase in voltage) and the osmotic gradient is reasonably linear. Though a control pulse which was iso-osmotic to the vesicle suspension measured 0 voltage, an extrapolated non-zero intercept is produced in Fig. 1. This is due to the presence of an osmotically insensitive component of the vesicle volume. The rate of H_2O movement is accelerated by increases in external osmotic pressure. The $t_{1/2}$ of this process ranges from 538 msec at 70 mosmol to 96.5 msec at 1484 mosmol.

H_2O Permeability

Fig. 2 shows the oscilloscope record of the rate of shrinkage following the application of a 138 mosmol osmotic gradient produced by 75 mM KCl at 24 °C. This shrinkage can be described by a single exponential. At this temperature the water movement has a rate constant (k_1) of 1.1 ± 0.09 sec^{-1} ($n=10$). The half maximal time of this shrinkage is 630 msec. An Arrhenius plot of this rate constant over a range of temperatures between 16 and 36 °C shows an activation energy of 13.9 ± 0.6 kcal mole^{-1} ($n=3$) (Fig. 3). This figure is typical of 3 different preparations.

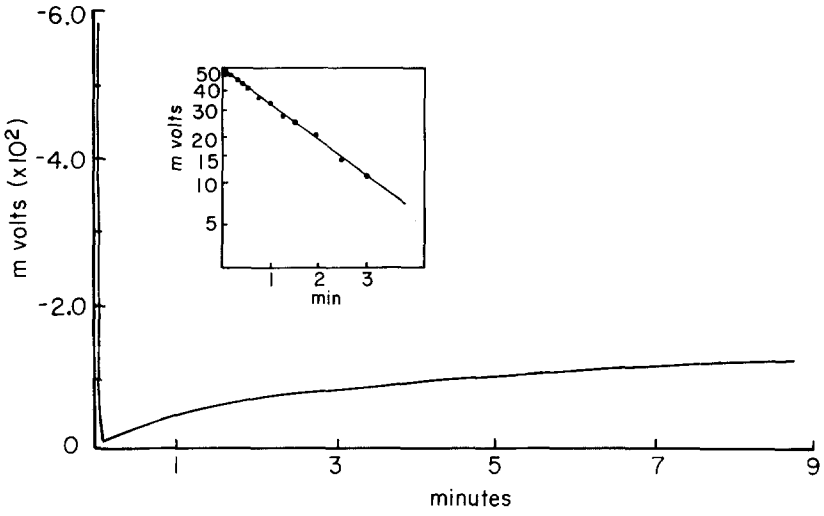

Fig. 4. KCl entry dependent reswelling at 25 °C. Gastric vesicles suspended in 294 mosmol sucrose medium were pulsed with 434 mosmol KCl + sucrose medium. Water transport in the fast signal is measured by an oscilloscope trace. The slower KCl dependent reswell is recorded on a Radiometer REC 61 servorecorder. Note the orientation of the y axis. The insert is a semilog plot of the reswell phase used to determine the faster rate constant, k_1 of the reswell process

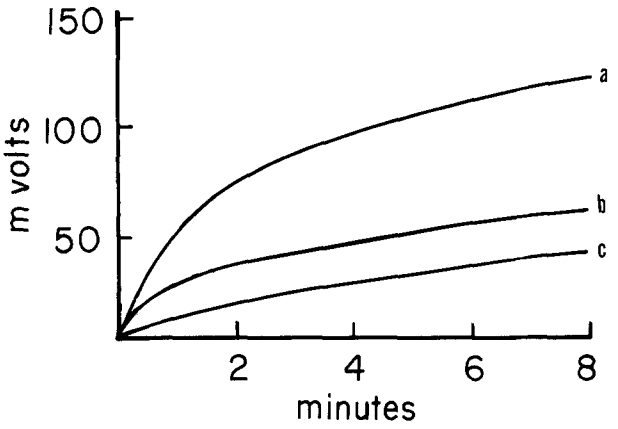

Fig. 5. Effect of ionophores on KCl entry. 4.5 μM valinomycin or 1.0 μg/ml^{-1} nigericin was added to both gastric vesicle suspension and hypertonic KCl medium. Their effect on the rate of reswelling was observed following exposure of the vesicle suspension to the hypertonic KCl solution. The top curve *a* represents reswell in the presence of 4.5 μM valinomycin and the middle curve represents reswell with 1 μg/ml of nigericin *b*. The control, in the absence of ionophores, is the bottom curve *c*. *See* Materials and Methods for details

Table 1. Effect of ionophores on KCl entry[a]

Ionophore	k_1 (sec^{-1}) $\times 10^{-3}$	k_1' (sec^{-1}) $\times 10^{-3}$	k_2 (sec^{-1}) $\times 10^{-4}$	% total amplitude (k_1+k_1')
None	7.75±0.48 (n=15)	–	1.94±0.23 (n=15)	9.0±0.64 (n=13)
Nigericin (1 μg ml^{-1})	15.7 ±3.7 (n=5)	6.76±0.74 (n=5)	1.33±0.21 (n=5)	9.9±1.6 (n=5)
Valinomycin (4.5 μM)	17.2 ±3.4 (n=3)	10.2 ±1.3 (n=2)	2.20±0.46 (n=3)	22.3±3.7 (n=3)

[a] The results listed are from several vesicle preparations which were used throughout this investigation. All refer to standard conditions which were a gradient of 138 mosmol KCl in 0.25 M sucrose, 2 mM $MgCl_2$, 2 mM PIPES/Tris, pH 7.4. The protein concentration after mixing was 0.037 mg ml^{-1}. The reaction temperature was 25.3 °C.

Ion Transport

In control vesicles the reswell accompanying KCl entry into the shrunken vesicles occurs in two distinct phases. This KCl entry, which is a necessary requirement for proton transport (Sachs et al., 1976) is seen as a decrease in the intensity of 90° scattered light. This reswelling phase for the initial 9 min is shown in Fig. 4. It can be seen that even after 9 min at 24 °C, only 17% of the original volume has been recovered. The reswelling curve can be fitted to a single exponential imposed upon a constantly sloping background. The rapid phase of the reswell process, shown in Fig. 4, accounts for only 9.8% of the total reswell signal. The determination of the rate constant, k_1, for this process is shown in the insert of this figure. As listed in Table 1, $k_1 = 7.75 \pm 0.48 \times 10^{-3}$ sec^{-1} (n=15). This signal has a $t_{1/2}$ of 1.49 min. The slower phase, plotted as the constantly sloping background, accounts for the majority of the reswell signal. With its rate constant, $k_2 = 1.94 \pm 0.23 \times 10^{-4}$ (n=15) (Table 1), the slower rate constant is more than an order of magnitude slower than the faster rate constant. These values have been typical for all membrane preparations which are relatively tight to K^+ and H^+ as seen by ΔpH formation experiments. Comparison of $t_{1/2}$ of the slower reswelling process ($t_{1/2}$=59 min) to that of $^{86}Rb^+$ equilibration ($t_{1/2}$=46 min) (Schackmann et al., 1977) indicates the similarity of the two processes. Since the scatter change measurement is taken within the first 15 min of the reswell process, it may be that the equilibration of KCl becomes more rapid as the time of incubation at room temperature increases. Both at

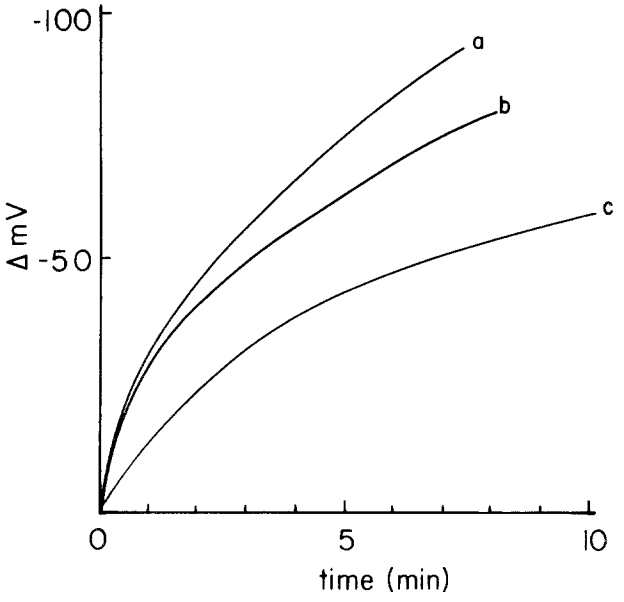

Fig. 6. Effect of aging on KCl entry. The effect of aging on the rate of reswelling after exposure of the gastric vesicles to hypertonic KCl was compared for vesicles of varying ages which were from the same original preparation. Vesicles were aged 2 days, (trace *c*), 7 days (trace *b*), and 11 days (trace *a*)

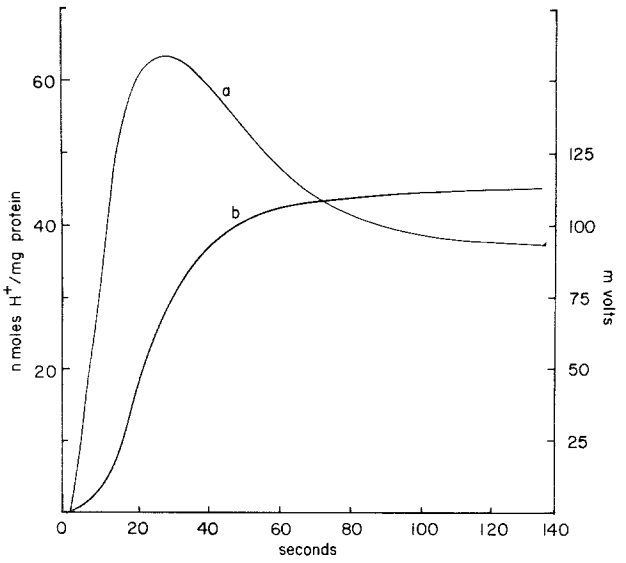

Fig. 7. Comparison of the development of ATP-dependent scatter (shrinkage) changes to the rate of development of ATP-dependent proton translocation. Trace *a:* measurement of proton translocation after addition of ATP to vesicles equilibrated in KCl, trace *b:* measurement of 90° light scatter intensity after addition of ATP to vesicles equilibrated in KCl. Temperature 23°, pH 6.1

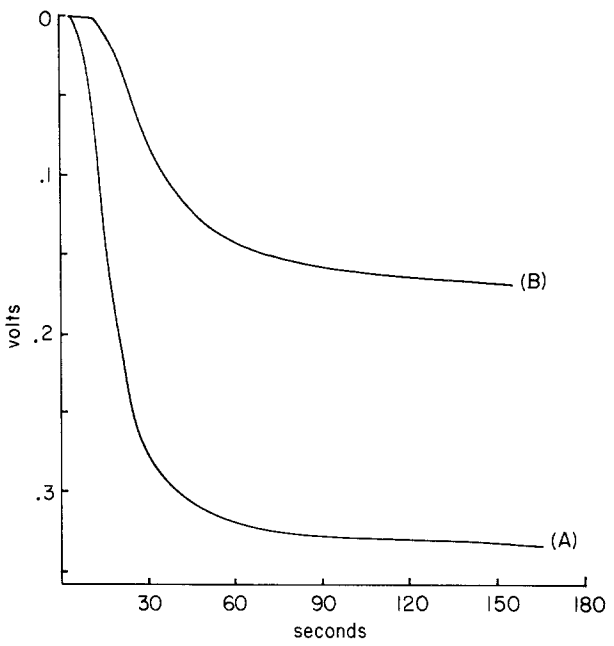

Fig. 8. Effect of buffer concentration of the development of ATP-dependent scatter (shrinkage) changes. Trace *A:* development of the scatter signal in gastric vesicles equilibrated in unbuffered 150 mM KCl, 2 mM $MgCl_2$; trace *B:* development of the scatter signal in gastric vesicles equilibrated in 2 mM PIPES/150 mM KCl and 2 mM $MgCl_2$. See Materials and Methods for details

Table 2. Rate constant for HCl leak from pH or scattering measurements[a]

	k_{leak} (26 °C)
ATP dependent 90° light scatter	$2.36 \pm 0.44 \times 10^{-2}$ sec^{-1} ($n=3$)
H^+ gradient dissipation	$2.42 \pm 0.10 \times 10^{-2}$ sec^{-1} ($n=3$)

[a] Measurement of HCl leak rates from aliquots of the same preparation. Equilibration conditions of 150 mM KCl, 2 mM $MgCl_2$ and 2 mM PIPES/Tris pH 6.1 were identical in both experiments. Transport was initiated by the addition of 0.8 mM Mg ATP, pH 6.1, at 0 time.

room temperature and at 37 °C, the signal returns to the original baseline. This return to baseline is also seen more rapidly with urea, a species therefore more permeant than KCl in these vesicles.

The effect of ionophores on KCl entry was studied since these substances have significant effects on ATP-dependent ion transport in gastric vesicles. As shown in Fig. 5, the rate of KCl uptake is sensitive to 4.5 μM valinomycin and 1 μg ml^{-1} nigericin. The addition of valinomycin (upper trace) or nigericin (middle trace) significantly accelerated the fast phase of uptake of KCl over that of the control (lower trace) in the absence of ionophores.

The effect of nigericin appears predominantly on the faster phase occurring within the initial 5 min of reswell. The effect of 1 μg ml^{-1} nigericin, listed in Table 1, results in two separable rate constants in the fast reswell phase: the faster $k_1 = 15.74 \pm 3.7 \times 10^{-3}$ sec^{-1} ($n=5$), and the slower $k'_1 = 6.76 \pm 0.74 \times 10^{-3}$ sec^{-1} ($n=5$) which is approximate-

ly that of control. The total amplitude of $k_1+k'_1$ is within the variance of control values. The initial phase of KCl-dependent reswell is also resolvable into two rate constants in the presence of 4.5 μM valinomycin. The faster, $k=17.2\pm3.4\times10^{-3}$ ($n=3$), is more than twice the control value, while the slower constant, $k'_1=10.2\pm1.3\times10^{-3}$ ($n=3$), is close to the control value. The sums of magnitude of the reswell processes represented by k_1 and k'_1 are approximately double those of the control value. Neither ionophore appears to significantly affect the slow reswell phase accounting for KCl equilibration into the majority of the vesicle population.

Recent transport studies have indicated an age-dependent change in Cl^- conductance pathways in the GI vesicle fraction (Rabon, Kajdos & Sachs, 1979). To test if age-dependent changes in ion conductance pathways are evident in the general vesicle population, the KCl entry phase was recorded at 25 °C under the standard osmotic gradient of 138 mosm. The initial KCl entry phases for variously aged vesicles from the same preparation are shown in Fig. 6. The vesicles were aged by 4 °C storage in the standard medium consisting of 0.25 M sucrose, 2 mM $MgCl_2$, 2 mM PIPES/Tris pH 7.4 and 0.075 mg ml^{-1} protein. The slowest initial rate of KCl entry is recorded in trace *c*, the 2-day-old material. This rate increases with advancing age. Trace *b* is the 7-day-old preparation, and trace *a* is the 11-day-old preparation. This trend of an age-dependent increase in the entry rate of KCl as shown in Fig. 6 is typical of two preparations.

Osmotic Effects of ATP

The addition of 0.8 mM ATP to gastric vesicles pre-equilibrated with KCl results in the uptake of H^+. From trace *a* of Fig. 7, it can be seen that this uptake process exhibits three phases, an initial rapid entry of H^+ into the intravesicular space with an overshoot, which is followed by a decrease to a steady state value. The substitution of SO_4^{--} for Cl^- abolishes this overshoot (Sachs et al., 1977). Because the AT-Pase supports a $H^+:K^+$ antiport that is electroneutral, the observed uptake of H^+ is due to this $H^+:K^+$ exchange. Since, in the continuing presence of ATP, efflux of H^+ at the zenith of the overshoot is not due to $H^+:K^+$ exchange, the H^+ leak must be through the loss of $H^+ + Cl^-$. This loss of intravesicular HCl would then result in a decrease of vesicular volume. Trace *b* of Fig. 7 is a measure of this osmotically induced shrinkage obtained as the ATP-dependent change in light scatter 90° to incident. It can be seen that under these transport conditions there is a characteristic delay phase lasting about 6 sec. After this delay phase, vesicle shrinkage increases to a maximum at a time when the pH gradient shown in trace *a* reaches the steady-state phase.

Addition of 1 μg ml^{-1} of either nigericin or gramicidin before addition of ATP prevents the formation of the pH gradient, as well as the ATP dependent scatter change. Similarly, SO_4^{--} substitution for Cl^- results in a loss of the scatter change following ATP addition (data not shown). This scatter change thus appears to depend on H^+ gradient formation and on the HCl leak component as seen in the overshoot phase of the H^+ transport process.

Fig. 8 indicates the buffer sensitivity of the ATP-dependent scatter change. Comparison of trace *a* (unbuffered) to trace *b* (buffered) indicates that an increase in the buffer capacity of the intravesicular space increases the initial delay phase and decreases the magnitude of the scatter change. With increased vesicular buffering a smaller transvesicular H^+ gradient would be anticipated, as well as a longer delay time to reach that gradient. Lower buffering would allow a more rapid establishment of a larger gradient. From these considerations, the absence of buffer permits the rapid establishment of a H^+ gradient. In turn, the leak of this gradient as HCl determines the magnitude of the 90° shrink signal.

Comparison of the magnitude of the ATP-induced shrinkage to the calibration curve of Fig. 1 indicates a range of ATP-induced shrinkage for 2 mM PIPES/Tris buffered and unbuffered vesicles from 7 to 14% respectively of the osmotically sensitive space (about 10% of the intravesicular space appears to be osmotically insensitive).

Table 2 is a comparison of the rate at which the proton gradient dissipates after proton uptake has been limited by the depletion of internal K^+. These experiments were performed on aliquots from the same incubation medium under identical conditions of temperature and ATP concentration. As indicated in Table 2, there is an equality between the rate constants representing the decay of the H^+ gradient and the formation of the scatter signal. Thus, scattering measurements give a rate constant of 2.36×10^{-2} sec^{-1} and the pH electrode trace gives a rate constant of 2.42×10^{-2} sec^{-1} under identical conditions, hence the leak rate of HCl is the same by both techniques.

Discussion

The use of osmotic techniques for gastric vesicles have produced a set of data which extend our previous knowledge of the passive permeability characteristics of these membranes.

In the case of this preparation, as for others, such as sarcoplasmic reticulum (Kometani & Kasa, 1978) or mitochondria (Tedeschi & Harris, 1955) there is a vesicle dead space, corresponding to a minimum volume that the vesicles can reach with increasing medium tonicity. In terms of water permeability, the preparations exhibited a single rate constant, 1.1 ± 0.09 sec^{-1} ($n=10$). Because of the closeness of fit of the water movement phase to a single exponential, it is unlikely that this apparent rate constant reflects two distinct vesicle populations in terms of size. It is likely that this single rate constant is determined by the average rate of water movement from a somewhat heterogeneous population of vesicles. The energy of activation for the permeation process was 13.93 ± 0.6 kcal mol^{-1} ($n=3$). This can be compared with activation energies ranging from 2.5 kcal/mol in lobster nerve (Nevis, 1958), 3.9 kcal/mol for the red cell (Jacobs, Glassman & Parpart, 1935); 4.1 kcal/mol for vasopressin treated toad bladder (Hays & Leaf, 1962), and 8.9 kcal/mol in cortical collecting duct (Al Zahid et al., 1977). These relatively lower activation energies have been interpreted as indicating non-lipid permeation pathways through the bilayer. Hence, the gastric vesicles apparently do not contain such pathways. The energy of activation of water permeability can be more directly compared with the activation energy of 12.7 kcal/mol for lipid bilayers (Price & Thompson, 1969) and 17.5 kcal/mol for butyramide in the cortical collecting duct (Al Zahid et al., 1977). It should be noted that in three preparations there was no evidence for a phase transition between 16 °C and 36 °C, the temperature range which encompasses an ATPase or H^+ transport activity transition (Chang et al., 1977). The H_2O permeability of these vesicles is of more than passing interest. Gastric secretion is only slightly hypertonic to plasma over a wide range of secretory rates and (Durbin & Helander, 1978) thus a mechanism must exist to equilibrate water across the secretory membrane at high secretory rates. This membrane has been localized by immunological techniques to the microvillar surface of the secretory canaliculus (Saccomani et al., 1978). Calculations have shown (Durbin & Helander, 1978) that lipid pathways for H_2O movement appear adequate to account for the solvent flow across the gastric mucosa, consistent, then, with the pathway for H_2O flow, deduced to be present in the vesicle preparation. P_w was about 10^{-4} cm/sec.

The KCl permeation properties of the gastric vesicles are germane to the H^+ pump mechanism of the gastric K^+ ATPase. According to the model developed from transport studies, there are two K^+ sites, a high affinity site involved directly in ATP hydrolysis and H^+ transport and a low affinity inhibitory site. The former is occupied only from the solution internal to the vesicle. Thus, KCl must enter the vesicle, or at least reach this site, for H^+ transport to occur. Prior studies (Sachs, 1977) have indicated that K^+ entry is largely nonconductive, but can be increased by valinomycin or nigericin (Ganser & Forte, 1973). The former ionophore increases ATPase activity and H^+ transport; the latter, as well as gramicidin, are more effective activators of the ATPase but H^+ transport is shunted in their presence. About 10% of the vesicle population showed a relatively rapid uptake of KCl. Since long preincubation times are required for maximum H^+ transport to be measured, this group of vesicles evidently is not the most significant population concerned with H^+ gradient formation. The addition of 4.5 μM valinomycin resulted in an initial KCl entry which could be resolved into two distinct phases, as compared to a single rate constant in the control. As indicated in Table 1, the faster rate constant describing K^+ entry was approximately double the initial rate constant of the control in the absence of ionophore. The second rate constant (k'_1) observed in the presence of valinomycin was approximately equal to that measured as the faster rate constant for the control. The fast reswell phase represents approximately 22% of the total shrink signal, twice the magnitude of that observed in the absence of ionophore.

These data are consistent with a K^+ conductance limitation for KCl entry in 22% of the vesicle population revealed by the addition of valinomycin. The compensating conductance could be for Cl^- or H^+. Aging the vesicles increased the rate of KCl penetration into the vesicles, as evidenced by the osmotic signal, and previous data have shown that a Cl^- conductance is produced in gastric vesicles by aging or proteolysis (Rabon, Kajdos & Sachs, 1979). Valinomycin in fresh gastric vesicles is also considerably less effective than gramicidin or nigericin (H^+:K^+ exchange ionophores) in stimulating K^+ dependent ATPase activity (Sachs, 1977). Again this is consistent with a limitation on valinomycin-dependent K^+ entry due to low membrane conductance for other ions.

The rate constant for KCl entry in the majority of the vesicles gives a $t_{1/2}$ of 59 min which can be compared to the $t_{1/2}$ of $^{86}Rb^+$ uptake of 46 min (Schackmann et al., 1977; Schackmann, 1976). This then raises the question as to the mechanism of stimulation of H^+ transport by valinomycin in vesicles which have not been preloaded with KCl (Sachs et al., 1976; 1977). Either the enhanced signal is due to a small fraction of the vesicle population, or, given the slow development of the valinomycin dependent H^+ transport signal, the osmotic technique is not

sufficiently sensitive to detect small changes in slow rate constants.

The effect of an obligatory exchange ionophore, nigericin, in increasing the rate of reswelling, implies that the H^+ lost from the vesicle interior was not as osmotically active as the K^+ ion which replaces it. A simple explanation is that the H^+ loss is from protonated positively charged sites. Such buffering sites are implied by the demonstration of a larger loss of H^+ from the external medium as compared to appearance of free H^+ in the internal vesicle space (Rabon et al., 1978*a*).

ATP has an effect on vesicle volume if the vesicles are loaded with KCl prior to the addition of ATP. Under these conditions, there is a characteristic time course of change of medium or internal pH. There is an initial rapid phase, reaching a maximum, and then a decay of the pH gradient to a steady state as long as the supply of ATP lasts. The initial phase can be modelled as rapid exchange of internal K^+ for external H^+. Since the H^+ leak in the vesicles exceeds the K^+ entry, this exchange must be followed by a loss of HCl from the vesicles until the H^+ leak and K^+ entry are exactly equal, establishing the steady state.

This loss of HCl should be accompanied by shrinkage of the vesicles, as indeed found with ATP addition. The time course of the shrinkage is coincident with the time course of the decay phase of the overshoot. SO'_4' substitution for Cl^- eliminates the overshoot and the ATP-induced shrinkage. Increasing intravesicular buffering, for example, by addition of PIPES/Tris, delays and reduces the shrinkage by presumably reducing free HCl concentration for a given quantity of K^+ extruded. A comparison of the rate constants for KCl entry (slow phase) and HCl leak (Tables 1 and 2) shows that, indeed, these rates are sufficiently different to account for the phenomenon, and that the steady state ΔpH, given an infinite supply of ATP, is not determined by pump capacity, but by the respective leak pathways. This suggests that measurement of gradient capacity of the pump in vesicular form does not allow conclusions to be drawn as to whether the H^+:K^+ ATPase alone can account for the physiological ΔpH of more than six units.

Moreover, if all the trapped H^+ were present as free HCl, the shrinkage would exceed the 15% determined experimentally, indicating that a considerable fraction of the transported H^+ is present as bound or buffered H^+. These data illustrate an additional requirement in modelling active pump phenomena in vesicles, namely, the maintenance of isotonicity.

Osmotic techniques, therefore, allow parameters of vesicle H_2O and solute transport to be analyzed which are not readily accessible by other techniques. Finally, it should be pointed out that, according to our model of H^+ secretion by these vesicles, namely a KCl symport mediated entry of salt, followed by an ATP-dependent H^+ for K^+ exchange, the osmotic force necessary to maintain flow of secretion in the intact cell depends on the K^+ gradient, cell-lumen, produced by the pump. This is one of the probable driving forces for water flow during gastric secretion.

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