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Kinetic Properties of the KCl Transport at the Secreting Apical Membrane of the Oxyntic Cell

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Summary. We have previously reported marked structural and functional differencces between (H^+/K^+)-ATPase-rich vesicles obtained from resting fundic gastric mucosa and those obtained from secreting tissue. The functional differences seem to arise from activation of a permeability pathway for K^+ salts in the vesicles originating from stimulated tissue. By providing the intravesicular K^+ needed for the ATPase-mediated H^+/K^+ exchange, this pathway facilitates formation of large pH gradients (J.M. Wolosin & J.G. Forte. *FEBS Lett.* **125**:208–212, 1981). We have now expanded our observations on the characteristics of the K-salt transport. The dependence of intravesicular acidification on the concentration of various anions or of K^+ was monitored using the rate of fluorescence quenching of the pH probe, acridine orange. Rates of acid accumulation followed the series $I^- > Br^- > Cl^- > NO_3^- > CH_3SO_3^-$ > isethionate > gluconate. The effects of exogenous ionophores on pH gradients suggested that K^+-salt transport is electroneutral. On the basis of the known properties of the H^+/K^+ pump, we devised a method by which the rates of unidirectional K^+ salt influx can be monitored via the resulting rates of H^+ accumulation. Accordingly, the dependence of these rates on the concentration of K^+ and Cl^- was studied and subjected to a kinetic analysis. It was found that the dependence follows that expected for an electroneutral K^+ and Cl^- transport system with apparent Michaelis constants equal to 15 ± 2 mM for K^+ and 46 ± 5 mM for Cl^-. It is concluded that intravesicular acid accumulation, believed to represent HCl secretion in whole tissue, results from the combined action of a KCl symport and the well known $(H^+ + K^+)$-ATPase. Though the calculated maximal transport capacity of the symport exceeds by several times the maximal transport capacity of the pump, at physiological concentrations of the ions both transport systems are expected to transport K^+ in opposite directions at similar rates. Implications of these observations to HCl secretion by intact tissue are discussed.

Key words HCl secretion · cotransport · oxantic cell · $(H^+ + K^+)$-ATPase · apical membrane · membrane transport

Introduction

The stimulated mammalian oxyntic mucosa is capable of secreting an isotonic HCl solution, imposing a 10^6 H^+ gradient across the luminal surface of the gastric epithelium. A large body of evidence identifies the oxyntic cell present in the glands of the fundic and main body areas of the stomach as responsible for this extreme secretory process (Bradford & Davies, 1950; Davenport, 1971; Berglindh, Di Bona, Ito & Sachs, 1980). In the last decade, with the advent of suitable biochemical and biophysical techniques, our knowledge of the molecular mechanisms involved has been significantly advanced. Ganser and Forte (1973) identified a K^+-dependent ATPase in purified light density microsomes derived from homogenates of oxyntic cells. The microsomes were subsequently shown to be capable of H^+ accumulation in the presence of K^+ at the expense of ATP (Lee, Simpson & Scholes, 1974) and it was established that H^+ accumulation was mediated by an electroneutral H^+/K^+ exchange pump (Sachs et al., 1976) which utilizes intravesicular K^+ and is currently known as $(H^+ + K^+$-ATPase.

The enzyme has been recently localized by immunochemical techniques to the apical surface of the oxyntic cell (Saccomani et al., 1979). It would be logical, then, to propose that the gastric microsomes are simply 'inside-out' membranes derived from the apical surface of the oxyntic cell so that their intravesicular volume corresponds to the canalicular and luminal space of the cell, and the extravesicular medium corresponds to the cell cytoplasm. The K^+ permeability in gastric microsomes, however, is very limited and unable to sustain intravesicular acidification (Lee & Forte, 1978). Efficient and sustained H^+ accumulation requires the presence of the exogenous K^+ ionophore valinomycin. The ionophore allows efficient K^+ (and Cl^-) internalization, then the ATPase

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pump exchanges the cation for hydrogen ion. In addition, concentrations of Cl^- larger than 100 mM are required; concentrations corresponding to those present in the cell cytoplasm (i.e., 20–40 mM Cl^-) are unable to promote rapid rates of H^+ accumulation (Lee, Breitbart, Berman & Forte, 1979). Clearly, K^+ and Cl^- permeabilities in gastric microsomes are inadequate to explain the acid secretory process. Since K^+ is an essential requirement for acid secretion (Sanders, Noyes, Spangler & Rehm, 1973) and since K^+ secretion accompanies H^+ secretion (Davenport, 1971; Sen, Tague & Ray, 1980), it seems highly possible that in the intact cell an endogeneous mechanism is responsible for the delivery of K^+ from cell to lumen.

We have recently reported the striking differences in distribution of K^+-ATPase in cell fractions derived from resting versus maximally stimulated gastric mucosa (Wolosin & Forte, 1981*a*). Stimulation induced a large decrease in the yield of gastric microsomes (50–70%) and the concurrent appearance of a new type of K^+-ATPase-rich membrane vesicles, larger and heavier than gastric microsomes. We have linked these membrane changes and K^+-ATPase redistribution with the known morphological transformations that occur during the secretory cycle of the oxyntic cell (Ito & Schofield, 1974; Forte, Machen & Forte, 1977) by proposing that gastric microsomes are directly derived from the endoplasmic tubulovesicular system of the resting oxyntic cell while the new membrane vesicles derive from the expanded apical membrane of the stimulated state. Subsequently, we demonstrated that the new membranes, which we call stimulation-associated (or s.a.) vesicles, display a high passive, and probably electroneutral K salt permeability. With this symport system, the vesicles can generate intravesicular acid through the ATP-driven exchange of H^+ for pre-internalized K^+ (Wolosin & Forte, 1981*b*, *c*). Some of the properties displayed by this symport indicate that its activity is under metabolic regulation implying that the system is active only in the stimulated cell. Recently, studies on isolated gastric glands have substantiated the existence of such a K-salt permeability pathway in the apical cell membrane (Malinowska, Koelz, Hersey & Sachs, 1981). In this report, we provide further evidence of the electroneutral character of the passive K^+ and Cl^- permeability system. We also present a method for the study of the kinetics of the symport and report some kinetic parameters determined by this method.

Materials and Methods

Membrane Preparations

Gastric microsomes and stimulation-associated (s.a). vesicles were isolated from the fundus and main body of rabbit gastric mucosae by the methods previously described (Wolosin & Forte, 1981*b*) with some modifications. Animals were fasted for 24 hr. Unstimulated mucosae were obtained by subcutaneous administration of metiamide (0.5 mg/kg body weight), stomachs were taken one hour later. For stimulated mucosae the animals were allowed to feed on staple food for 10 min followed by one subcutaneous injection of chlorophenearamine maleate (1 mg/kg body weight) and two histamine injections (0.2 mg/kg) 15 min and 30 min later. Stomachs were taken 20 min after the last injection. The oxyntic glandular regions of the mucosa, cleaned of mucus and surface cells by blotting and light scraping, were separated from the muscle layer, minced with surgical scissors and homogenized in 30 volumes of ice-cold buffer (125 mM mannitol, 40 mM sucrose, 1 mM EDTA, 5 mM piperazine N,N-1-bis (2 ethanesulfonic acid), pH 6.7, by 15 passes at 200 rpm through a Teflon piston homogenizer (Potter-Elvehejm). The homogenate was centrifuged twice at 2000 rpm × 4 min in a SS-34 Sorval Rotor. The pH of the supernatant was raised to 7.4 by addition of 1 M Tris base and centrifuged twice at 11,000 rpm for 11 min. The precipitates were combined to form the crude mitochondrial pellet and the supernatant was centrifuged at 20,000 rpm × 90 min yielding a crude microsomal pellet. The final supernatant was discarded. To obtain s.a. vesicles, the crude mitochondrial pellet derived from stimulated tissue was suspended in a 300 mM sucrose-5 mM Tris buffer, pH 7.4, and fractionated in a 9–15% Ficoll step gradient built in the same medium at 27,000 rpm for 6 to 16 hr using a Beckman SW27 rotor. The material in the 9–15% interface was recovered and freed from polymer by dilution in 20 volumes of 300 mM sucrose and recentrifugation. The final material with a K^+-ATPase specific activity of around 40 μmol P_i/mg × hr was resuspended in 1 M sucrose and stored at −20 °C until use. Gastric microsomes were obtained by fractionating the crude microsomal pellet derived from unstimulated mucosae in a 22–27–33% sucrose step gradient. The purified microsomes were recovered from the 22–27% sucrose interface and stored at −20 °C. The preparation has typical specific activities for K^+-ATPase of 60–70 μmol P_i/mg × hr. ($K^+ + H^+$)-ATPase activity was assayed as previously described (Wolosin & Forte, 1981*a*). Protein was determined according to Bradford (1976).

Uptake of ^{36}Cl by s.a. Vesicles

Vesicles (500 μl) were suspended in 250 mM sucrose at 2 mg protein/ml and mixed with an equal volume of a solution containing 250 mM sucrose, twice the desired final concentrations of the other solutes and 2 μCi ^{36}Cl. Both solutions were pre-equilibrated to the desired incubation temperature. At various times, 70-μl aliquots were added to 5 ml of ice-cold stopping solution (25 mM K gluconate) which was immediately filtered through Sartorius hydro-

phobic rim filters (0.45 μm). The filters were washed twice with 5 ml of stopping solution and the trapped radioactivity was determined by liquid scintillation.

Measurements of Proton Accumulation Rates by the Fluorescence Quenching of Acridine Orange

Vesicular transport of H^+ was assessed by the fluorescence quenching of the probe acridine orange. All fluorescence measurements were performed using a Perkin Elmer MPF44A spectrofluorometer. Earlier studies have shown that the quenching of fluorescence is proportional to the amount of probe accumulated in response to a pH gradient (Lee & Forte, 1978). The actual ΔpH achieved in s.a. vesicles and gastric microsomes is usually determined by calibration against artificially generated pH gradients using the Tris-succinate method (Jagendorf & Uribe, 1966). This method, however, does not provide direct insight into the relationship between rate of proton accumulation and rate of fluorescence quenching. An approach to provide a semiquantitative analysis of that relationship is presented below. First, (a) we analyze the relationship between the rate of net proton transfer from extra- to intravesicular space and the rate of accumulation of a monoamine dye probe, then (b) we discuss the effect of acridine orange accumulation on total sample fluorescence, and finally (c) we describe the use of fluorescence quenching rates to estimate the relative rates of H^+ pumping by an ATPase pump whose activity is independent of the generated pH gradient.

a) The distribution of a weak base between the intra- and extravesicular space of a membrane vesicle suspension is influenced by the ΔpH between both compartments. For a monamine which is highly permeable in its unprotonated form (A) but impermeable as the cationic species (AH^+) and whose constant of dissociation, K_a (where $K_a = [A][H^+]/[AH^+]$), is much smaller than the intra- and extravesicular H^+ concentrations (i.e., $K_a \ll [H]_i$, $[H]_o$), it holds that (Lee & Forte, 1978)

$$[H^+]_i/[H^+]_o = [AH^+]_i/[AH^+]_o = \frac{AH_i^+}{AH_o^+} \cdot \frac{v_o}{v_i} \tag{1}$$

where v is the compartment volume and the subscripts o and i refer to the respective extra- and intravesicular compartments. If $v_i \ll v_o$, for $[H^+]_i = [H^+]_o$, the amount of extravesicular protonated amine practically equals the total amine (TA) present in the system (i.e., $AH_i^+ \sim 0$). Hence for experiments carried out in well-buffered media ($[H_o^+]$ = const), Eq. (1) can be rewritten $H_i^+ = [H^+]_o v_o AH_i^+/(TA - AH_i^+)$. Taking the derivative,

$$dH_i^+ = [H^+]_o v_o \frac{TA}{(TA - AH_i^+)^2} dAH_i^+. \tag{2}$$

Under conditions when $AH_i < 0.05\,TA$, e.g., near initial rate of change,

$$dH_i^+ \approx [H^+]_o \frac{v_o}{TA} d[AH^+]_i. \tag{3}$$

Thus, provided H_o^+ is kept constant (e.g., by a well-buffered medium), the initial change in extravesicular concentration of monoamine is roughly proportional to the change in intravesicular H^+ concentration.

Protons transferred into an intravesicular space (H_T^+) will distribute in three distinguishable states: i) as free protons (H^+), ii) bound to permeable amines (i.e., AH^+), and iii) bound to fixed H^+ acceptors (BH), thus

$$dH_T^+ = dH_i^+ + dAH_{i,T}^+ + dBH \tag{4}$$

where $AH_{i,T}^+$ is the total amine (free and bound) accumulated in the vesicle, hence $dAH_{i,T}^+ = -dAH_o^+$. If for each mole of free amine in solution n moles become bound to sites in the membrane, $dAH_{i,T}^+ = (1+n)dAH_i^+$. Under the conditions $AH_i^+ < 0.05\,TA$, Eq. (4) can be arranged, and after inserting Eq. (3) and replacing $d[AH^+]_i v_o$ by dAH_i^+, we have

$$dH_T^+ = (1 + (1+n)\,TA/H_o^+ + dBH/dH_i^+)\,dH_i^+. \tag{5}$$

Thus if dBH/dAH_i^+ is either constant or very small over the range in question ($AH_i^+ < 0.05\,TA$), then dH_i^+ will be proportional to the rate of H^+ accumulation and considering Eq. (3) and the relationships between dAH_i^+, $dAH_{i,T}^+$ and dAH_o^+ we obtain

$$dH_T^+ = -\text{const}\, dAH_o^+. \tag{6}$$

b) A convenient way to monitor the changes in extravesicular amine concentration is with the use of fluorescent acridine amines. The fluorescence of these dyes has a strong negative dependence on the concentration and is completely quenched by binding to negative sites (Lee, Quintanilha & Forte, 1976). The relationship between the fluorescence change, dF/dt, and the change in extravesicular dye with uptake can be written in a general form as

$$\frac{1}{AH_o^+} dAH_o^+/dt = -\frac{\gamma}{F} dF/dt \tag{7}$$

where γ is a function expressing the proportional decrease in total sample fluorescence resulting from each additional AH^+ molecule internalized. When each accumulated molecule completely loses its fluorescence then γ is maximal and equals 1; when no decrease in fluorescence is observed, $\gamma = 0$. For a nonbinding dye, γ will approach 1 only after enough dye has accumulated to produce the self-quenching concentration effect (Lee & Forte, 1978).

In the case of acridine orange, however, Lee and Forte (1978) have demonstrated that the extent of dye accumulation is more than 10 times larger than the accumulation expected on the basis of Eq. (1). This was interpreted as the result of extensive binding (i.e., $n > 10$) of the accumulated dye to intravesicular negative sites. It was also shown by correlating fluorescence quenching and extravesicular dye disappearance that, even for low pH gradients, conditions equivalent to $\gamma = 1$ occur. Providing that the expression dBH/dH_i^+ behaves independently of H_i^+ over the H_i^+ range considered, a hypothesis that is subject to experimental testing, measurements of dF/dt will provide an accurate relative measure of dH_T^+/dt. In practice, dF/dt may display a time lag with respect to acid accumulation. This lag will occur if the intravesicular pH at the start of the experiment is higher than that of the medium (since amine dye accumulation will not occur until $pH_i^+ < pH_o^+$), or if a certain intravesicular concentration of dye is indeed necessary to produce the required $\gamma = 1$ value. Experimentally, we have found it convenient to pre-equilibrate the membranes at one pH unit lower than that of the medium so as to promote a small accu-

mulation of dye prior to the initiation of the ATP-dependent transport of H^+.

c) In the particular case studied here, H^+ accumulation is the result of a constant pumping mechanism, the so-called (H^+/K^+)-ATPase (ΔJ_p). At least for moderate pH_i values the activity of this pump seems to be independent of changes in $[H^+]_i$ (Lee et al., 1979). This is not surprising considering the extreme pH gradients that the enzyme can generate in the intact tissue. Because the assay of fluorescence quenching requires pre-establishment of some pH difference at the time measurements are made, the contribution of any existing H^+ 'leak' through dissipative pathways (L) to the net proton gain (dH_T^+/dt) should be taken into consideration, hence

$$dH_T^+ = J_p\,dt - L[\Delta H]\,dt \tag{8}$$

where it is assumed that over the short period of time considered $L(\Delta H^+)$, which is among other things a function of the H^+ gradient across the membrane (Lee et al., 1979), can be taken as a constant. To obtain $L(\Delta H^+)$, the pump is stopped after a ΔH has been established, then $J_p = 0$ and

$$L_{(\Delta H^+)} = -(dH_T^+/dt)^{J_p=0}.$$

Utilizing Eqs. (6) and (7) and rearranging terms, Eq. (8) assumes the final form

$$J_p = -\text{const}[(dF/dt) - (dF/dt)^{J_p=0}] = \text{const}(\Delta\, dF/dt). \tag{9}$$

Materials

Acridine orange was from Eastman Kodak. Chemicals were of highest purity available. Gluconate salts were prepared by titrating solutions of D-gluconic acid lactone with either KOH or N-methylglucamine (NMG) to pH 7.0. ^{36}Cl was purchased from New England Nuclear.

Results

Functional Properties of Stimulation-Associated (s.a.) Vesicles

When s.a. membranes are incubated in K^+ salts in the presence of Mg – ATP, a large uptake of acridine orange, corresponding to pH gradients over 4 units (Wolosin & Forte, 1981*b*), is observed (Fig. 1*A*). Unlike the case for gastric microsomes, which is depicted in Fig. 1*B*, acid accumulation is not dependent on the K^+ ionophore, valinomycin. While valinomycin does not induce a significant change in either rate of accumulation or maximal accumulation by s.a. vesicles, it has a dramatic effect on the rate of H^+ gradient dissipation induced by the protonophore, CCCP, suggesting that the endogeneous mechanism(s) for K^+ internalization in s.a. vesicles differs from the valinomycin-type mechanism.

The pattern of accumulation for each one of the K^+ salts tested can be analyzed in terms of

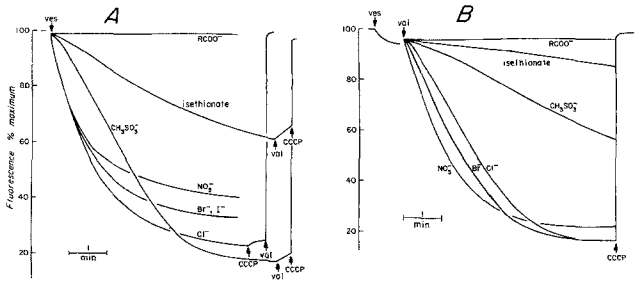
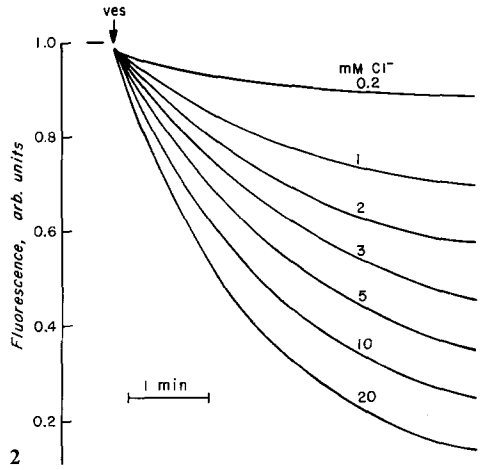


Fig. 1. Comparison of patterns of intravesicular acidification in stimulation-associated vesicles (*A*) and gastric microsomes (*B*). Development of pH gradients was monitored by the quenching of fluorescence of acridine orange. Experiments were started by addition of a small aliquot of vesicles (*ves*, 50 μg protein) to a medium consisting of (mM) 150 K^+ salt, 10 Na(TES), 0.1 EDTA, 1 $MgSO_4$, 1 NaATP and 4 μM acridine orange, pH 7.05. The various anions are indicated on the Figure. $RCOO^-$ is either acetate, gluconate or maleate. Other additions, where indicated by arrows, are 10^{-5} μM valinomycin (*val*) and 10^{-5} μM CCCP. (TES = N-tris[hydroxymethyl]methyl 2 amino (ethane sulfonic acid)

rates of intravesicular acidification and/or maximal pH gradient achievable. Because the latter is a combined function of the rate of proton accumulation and proton dissipation, its analysis is rather complex and will be resumed in the Discussion section. The initial rate of fluorescence quenching is largely determined by the rate of proton pumping (since H^+ dissipation is small for small H^+ gradients). As such, it seems to provide a satisfactory experimental basis to perform a comparative analysis of the functional similarities and differences between s.a. vesicles and valinomycin-complemented gastric microsomes seen with the various K^+ salts. Halogens and nitrate promote fast H^+ accumulation in both cases, but it is apparent from Fig. 1 that the rate of accumulation decreases in gastric microsomes with the series $NO_3^- > Br^- > Cl^-$, while the initial rates are indistinguishable for these anions in s.a. vesicles. Relative to these anions the sulfonates seem to elicit higher rates of accumulation in s.a. vesicles than in gastric microsomes, with methyl solfate > isethionate. Organic ions are completely incapable of eliciting any noticeable H^+ accumulation in either vesicular type. An interesting observation, for which a tentative explanation is given in the Discussion section, is that the maximal accumulations achieved in s.a. vesicles with NO_3^-, Br^-, Cl^- and $CH_3SO_3^-$ seem to be inversely proportional to the initial rates of accumulation for these anions in gastric microsomes.



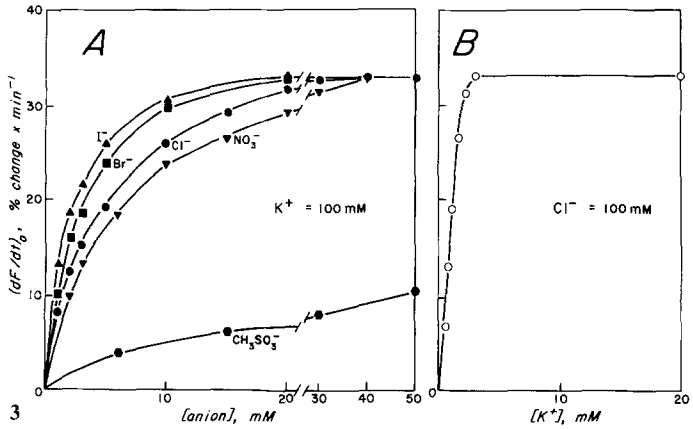


Fig. 2. Time course of fluorescence quenching in s.a. vesicles for various Cl^- concentrations. Experiments were performed as described in Fig. 1 in a medium consisting of (mM) 100 sucrose, 0.5 $MgSO_4$, 10 NaTES, pH 7.05, 1 NaATP, 4 μM acridine orange and 100 K^+ gluconate which was replaced as necessary by KCl in order to achieve the concentrations of Cl^- as noted in the Figure

Fig. 3. The rate of fluorescence quenching in s.a. vesicles as a function of the concentration of various anions (*A*) or as a function of K^+ (*B*). *A*. Anions were increased by an equivalent replacement for gluconate; K^+ was maintained at 100 mM. *B*. K^+ was increased by an equivalent replacement for choline with Cl^- maintained at 100 mM. The rate of fluorescence quenching at $t=0$ is looked upon as a relative measure of the actual rate of H^+ accumulation

Another important functional feature observed in s.a. vesicles is that, unlike the case for gastric microsomes (Lee et al., 1979), the K^+ or anion concentrations in the extravesicular medium can be greatly reduced before a decrease in the rate of H^+ uptake or the magnitude of the final pH gradient is visible. Figure 2 shows how the time course of fluorescence quenching depends on the concentration of Cl^-. As the concentration of anion is raised, the rate of accumulation and maximal uptake increase but both reach saturation around 20 mM Cl. The dependence of the rates of quenching on the concentration of various anions is depicted in Fig. 3*A*. When the concentration of anion is below 10 mM, differences in the rate of H^+ accumulation for the various anions become visible and follow the series $I^- > Br^- > Cl^- > NO_3^-$. The dependence of dF/dt on the concentration of K^+ is even sharper than that for the various anions (Fig. 3*B*).

The results above are conveniently explained on the basis of a high passive permeability in s.a. vesicles towards KCl and other K salts. This is demonstrated in Fig. 4*A* where rapid uptake of ^{36}Cl is shown for KCl in contrast to choline Cl. These experiments were carried out at lowered temperatures in order to permit a more accurate evaluation of the kinetics of uptake. At physiological temperatures the uptake is extremely fast (Fig. 4*B*) with a half-time for equilibration of about 10 sec. Increasing the co-ion concentration resulted in equilibrium ratios for Cl_i/Cl_o greater than 1. In gastric microsomes, K-salt permeation is slow and is accelerated four- to sevenfold by addition of valinomycin (Schackmann, Schwartz, Saccomani & Sachs, 1977; Lee et al., 1979; Wolosin & Forte, 1981*b*).

From the properties of s.a. vesicles described above, the following observations can be made: a) the valinomycin-induced K^+ influx is small compared to the intrinsic K^+ influx, and b) the valinomycin-induced K^+ influx is much larger than the endogenous conductive K^+ influx, which in the presence of CCCP seems insufficient to generate rapid gradient dissipation by K^+/H^+ conductive exchange. These observations, coupled with the high passive Cl^- permeability, lead to the conclusion that s.a. vesicles incorporate a passive K^+ salt electroneutral symport which generates large rates of K^+ (and corresponding anion) influx. The resulting intravesicular concentrations of K^+ appear to be sufficient to promote maximal ATPase-mediated H^+/K^+ exchange over a broad range of extravesicular K^+-salt concentrations. To postulate that K^+ enters s.a. vesicles through an electroneutral KCl pathway leads also to the conclusion that the conductive Cl^- permea-

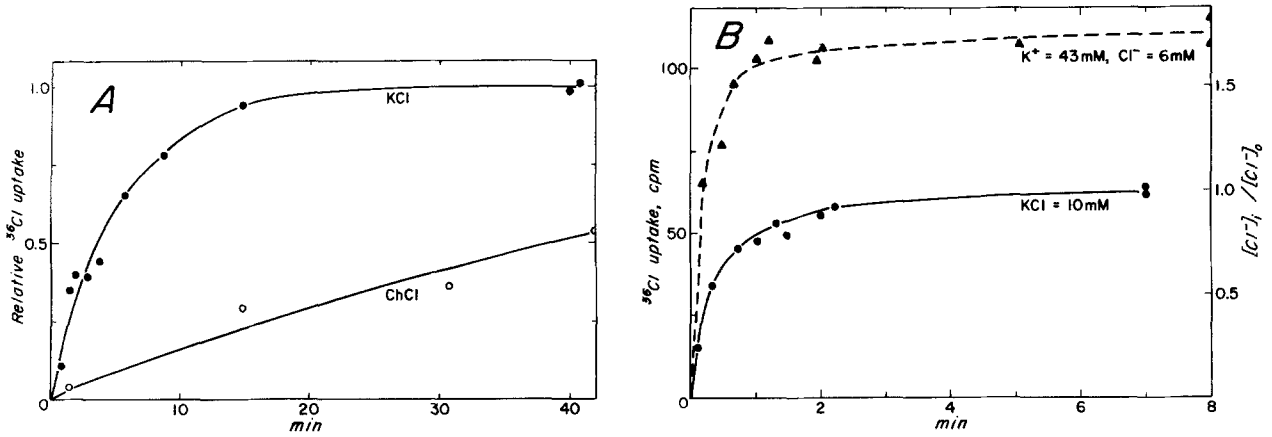


Fig. 4. Penetration of ^{36}Cl into s.a. vesicles. Isotope influx was measured as described in Materials and Methods. *A*: Experiments at 12 °C. The incubation medium contained 250 mM sucrose, 7.5 mM TrisTES (pH 7.2) and either 25 mM $K^{36}Cl$ (●) or 25 mM choline ^{36}Cl (○). *B*: Experiments at 36 °C. The incubation medium contained 250 mM sucrose, 7.5 mM TrisTES, pH 7.2, and either 10 mM $K^{36}Cl$ (●) or 43 mM K gluconate + 6 mM choline ^{36}Cl (▲). The ratio Cl_i^-/Cl_o^- in this latter case was calculated assuming that the intravesicular volume was not different than that in the case of 10 mM $K^{36}Cl$

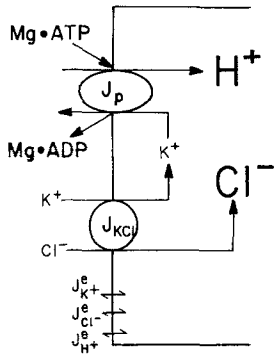


Fig. 5. Proposed functional model for the s.a. vesicles. KCl enters rapidly through an electroneutral KCl symport (J_{KCl}); K^+ is then recycled in exchange for H^+ through the ATP-driven pump (J_p); the net effect is HCl accumulation. In order to accommodate the observations made with various ionophores, it must be postulated that the fluxes through the electroneutral pathways (J_{KCl} and J_p) are normally much larger than the fluxes through any of the possible conductive pathways (J^e)

bility of the membrane is relatively small. Otherwise, in the presence of a protonophore a significant gradient dissipation should take place by the electrically coupled co-efflux of H^+ and Cl^-. A similar consideration for the case of acid accumulation in the presence of valinomycin (and no CCCP) allows us to conclude that the intrinsic H^+ permeability of the membrane is low when compared to the rate of H^+ accumulation, and hence to the rate of electroneutral influx of KCl.

The preceding conclusions are schematically summarized in Fig. 5. KCl is accumulated in the intravesicular space (corresponding to the luminal space in the intact cell); K^+ is then recycled by the MgATP-dependent pump in exchange for H^+, resulting in the net accumulation of HCl. All possible electrodiffusive leak pathways (J^e) are considerably smaller than electroneutral pathways (J_p, J_{KCl}).

The Relationship between the Rate of KCl Influx and the Rate of H^+ Accumulation at Steady State

Having established that in the s.a. membrane an electroneutral transport system co-exists with the electroneutral $(K^+ + H^+)$-ATPase pump, we sought to study the kinetic properties of the newly discovered system. Accurate measurements of kinetic parameters by conventional radioisotopic techniques seemed extremely difficult, given a) the short half-times for isotopic equilibration described above, and b) the fact that the purified preparation might contain an unknown fraction of membrane vesicles not derived from the oxyntic cell apical membrane. Alternatively, as will be shown below, acid accumulation can be used under certain conditions, as a measure of the unidirectional influx of KCl and hence serve to estimate kinetic parameters of the KCl transport system.

As depicted in Fig. 5 the rate of H^+ pumping at fixed extravesicular pH depends on the intravesicular concentration of K^+, $[K^+]_i$, and the extravesicular concentration of MgATP. Assuming a simple, random bireactant system for the activated enzyme-substrate complex, the pump activity could be represented by

$$J_p = n\,\text{ATPase} = \frac{J_p^{\max}[\text{MgATP}][K^+]_i}{(K_p^{\text{MgATP}}+[\text{MgATP}])(K_p^{K}+[K^+]_i)} \quad \text{(I)}$$

where J_p is the rate of the H^+/K^+ exchange pump, $J_p^{\max}$ is the maximal pump velocity, K_p^K and K_p^{MgATP} are the respective Michaelis constants for K_i^+ and MgATP, and n is the number of moles of H^+ exchanged for K^+ per mole of ATP hydrolyzed (ATPase). For saturating concentrations of MgATP the rate of H^+ pumping becomes solely dependent on $[K^+]_i$. This will vary according to

$$v_i \frac{d[K^+]_i}{dt} = J_{\text{inf}} - J_p - J_{\text{eff}}$$

where J_{inf} and J_{eff} are the unidirectional influx and efflux, respectively, through the electroneutral KCl symport and v_i is the internal volume. Upon establishment of a steady state, $d[K^+]_i/dt = 0$, and

$$J_p = J_{\text{inf}} - J_{\text{eff}}. \quad \text{(II)}$$

At constant [MgATP], according to Eq. (I), for any concentration of K^+ and/or Cl^- in the medium for which the resulting pumping rate is equal to or smaller than half of the maximal rate of pumping $\left(\text{i.e., } J_p \leqq \frac{J_p^{\max}}{2}\right)$, then $[K^+]_i \leqq K_m^K$. Wallmark et al. (1980) have shown that K_p^K is about 0.2 mM. Because this concentration of K^+ is, in physiological terms, very low, we considered the possibility that for $J_p \leqq \frac{J_p^{\max}}{2}$, $J_{\text{inf}} \gg J_{\text{eff}}$ and therefore that

$$J_p \simeq J_{\text{inf}}. \quad \text{(III)}$$

Hence, in certain ranges of extravesicular concentrations of K^+ and Cl^-, it is possible that J_p will be equivalent to the zero-trans unidirectional influx of KCl through the symport system. Since the J_{eff} is treated as a nonmeasurable unknown, the ultimate test for the validity of the assumption will be the consistency of measurements of J_p with J_{inf} values calculated from a suitable cotransport model.

A Linear Relationship between the Rate of H^+ Accumulation and the Rate of Fluorescence Quenching of Acridine Orange

The formation of pH gradients have conveniently been measured in small vesicles by indirect methods such as the accumulation of permeable amines. In Materials and Methods, we have analyzed the relationship between the rate of H^+ accumulation (dH_T^+/dt) and the rate of quenching of fluorescence (dF/dt) of acridine orange. It was shown that by measuring dF/dt at appropriate H^+ and by correcting for the contribution of H^+ leak through dissipative pathways, $L(\Delta H^+)$, estimates of J_p might be obtained. A typical experiment to determine (dF/dt) and $(dF/dt)^{J_p=0}$ is presented in Fig. 6*A*. The subindex *o* points to the fact that dF/dt was taken a few seconds after vesicle addition. As pointed out in Materials and Methods, the

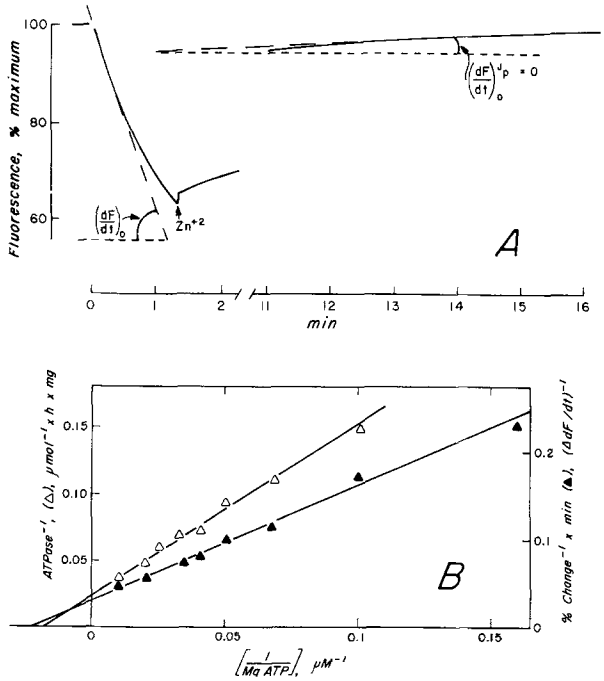


Fig. 6. Evaluation of the experimental relationship between $(\Delta dF/dt)_o$ and J_p. *A*: The method used to obtain $(\Delta dF/dt)_o$. Experiments were started by addition of vesicles pre-equilibrated at pH 6.0 to the desired medium and the rate of fluorescence quenching $(dF/dt)_o$ was measured. After stopping the H^+ pump by the addition of 2 mM $ZnSO_4(Zn^{2+})$, the gradient was allowed to dissipate and the rate of more fluorescence surge $(dF/dt)_o^{J=0}$ was measured. $(\Delta dF/dt)_o$ is obtained by algebraic subtraction of both measurements. *B*: Lineweaver-Burk plots of the dependence of K^+-ATPase activity and $(\Delta dF/dt)_o$ on the concentration of MgATP. Experiments were performed in a medium containing (mM) 75 KCl, 10 NaPipes, 1 Na-ATP plus the indicated amounts of MgATP. Values for $(\Delta dF/dt)_o$ were obtained as described in *A* using 30 μg protein/ml and 4 μM acridine orange. ATPase activities were measured by incubating vesicles (10 μg/ml) for 10 min in the control medium described above or in a medium in which KCl was replaced by choline Cl. K^+-ATPase activity was taken as the difference in ATPase activity between K^+ and choline media. [Pipes = Piperazine N, N′ bis (methane sulfonic acid)]

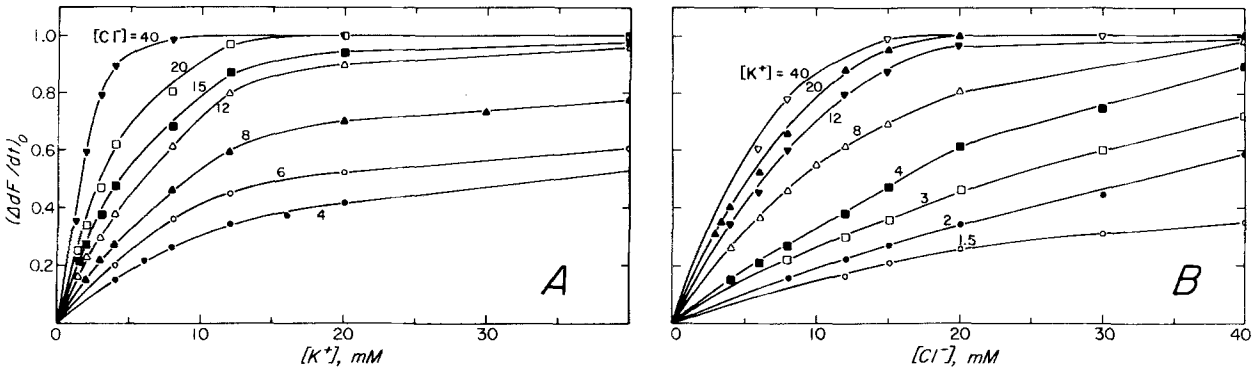


Fig. 7. The dependence of $(\Delta dF/dt)_o$ on the concentrations of Cl^- (*A*) and K^+ (*B*). $(\Delta dF/dt)_o$ was measured as described in Fig. 6*A*. The assay medium consisted of 10 mM TrisTES, 50 μM EDTA, 0.5 mM $MgSO_4$, 0.5 mM NaATP, 160 mM sucrose (pH 7.2) plus combinations of K-gluconate, NMG−Cl and NMG-gluconate required to achieve the desired concentrations of K^+ and Cl^- with the total salt concentration maintained at 60 mM. The correction for the contribution of dissipative leak, $(dF/dt)_o$, amounted to less than 4% of maximal value of $(dF/dt)_o$. *A*. The dependence on Cl^- is plotted at various fixed concentrations of K^+. *B*. The dependence on K^+ is plotted at various fixed concentrations of Cl^-.

buffer capacity in the intravesicular volume is an unknown element on which linear relations between J_p and $(\Delta dF/dt)_o$ will depend. It becomes necessary then to perform an experimental test of Eq. (9). In order to do this, it is necessary to change the J_p as an independent variable in a known and controllable way. For this purpose we exploited the functional properties of the $(K^+ + H^+)$-ATPase. From Eq. (I), at saturating K^+ concentration

$$J_p = n\text{ATPase} = \frac{J_p^{\max}[\text{MgATP}]}{K_p^{\text{MgATP}} + [MgATP]}. \qquad \text{(IV)}$$

Thus, J_p can be changed by varying the concentration of MgATP, and values obtained can be compared with a parallel set of measurements of ATPase activity. The results of such experiments are given in Fig. 6*B*. A double reciprocal plot of $(\Delta dF/dt)_o$ and [MgATP] produced a straight line demonstrating Michaelis-Menten kinetics and yielding a K_p^{MgATP} of 44 μM in good agreement with the value of 55 μM obtained from the direct K^+-ATPase measurements. This leads to the conclusion that

$$(\Delta dF/dt)_o = C_1 \times J_p. \qquad \text{(V)}$$

The Experimental Relationship between the Rate of H^+ Accumulation and the K^+ and Cl^- Concentration

From Eq. (I), we can see that J_p can also be changed by varying $[K^+]_i$ while keeping [MgATP] constant. $[K^+]_i$ will depend, in turn, on the extravesicular concentration of K^+ and/or Cl^-. In this case, by combining Eqs. (III) and (V), and adhering to the limiting condition that the pump rate, as reflected in $(\Delta dF/dt)_o$, is equal to or less than 1/2 of the maximal pump rate,

$$(\Delta dF/dt)_o \simeq C_1 J_{\text{inf}}. \qquad \text{(VI)}$$

The dependence of $(\Delta dF/dt)_o$ on the concentration of K^+ or Cl^- at various constant concentrations of the co-ion was studied. Results of these studies are depicted in Fig. 7. [The values of $(\Delta dF/dt)_o$ are presented as the fraction of the maximal $(\Delta dF/dt)_o$. In this relative unit system, $J_p^{\max} = 1$.] An interrelationship between K^+ and Cl^- is readily evident. As the concentration of one ion is raised, the concentration of co-ion that is required to achieve a certain rate of fluorescence quenching decreases. In the past, to explain the lack of effect of vesicle preincubation in KCl medium on the rates of H^+ uptake we have postulated that the maximal rate of KCl influx into the s.a. vesicle exceeds the maximal rate of ATP-driven H^+/K^+ exchange (Wolosin & Forte, 1981*b*). The sharp decrease in the K^+ concentration required to achieve saturation of the rates of H^+ uptake at high Cl^- concentrations (and vice versa) seems consistent with that postulate. The validity of Eq. (VI) could now be tested by confronting the experimental results for $(\Delta dF/dt)_o$ in Fig. 6 with an explicit expression for J_{inf}.

Kinetics of Unidirectional Flux through a 1:1 Obligatory Cotransport System

A random sequential binding model for the unidirectional influx under zero-trans con-

ditions through a 1:1 K^+ and Cl^- cotransport system (J_{ct}) is shown in Fig. 8. The binding constants for K^+ and Cl^- are given by k^+ and k^-, respectively; α is the extent to which binding of one of the ions can modify the affinity for the other; and κ is the rate constant for translocation of the fully loaded carrier. The general equation describing the flux (J_{ct}) for such a bi-substrate system, along with a detailed description of its kinetic properties, is available in the text by Segel (1975). In the context of the terms and model of Fig. 8, we have

$$J_{ct}=\frac{J_{ct}^{max}[K^+][Cl^-]/\alpha k_a^+ k_a^-}{1+[K^+]/k_a^+ + [Cl^-]/k_a^- + [K^+[[Cl^-]/\alpha k_a^+ k_a^-} \quad \text{(VII)}$$

where k^+, k^- are the apparent Michaelis constants for K^+ and Cl^-, respectively, in the absence of co-ion. The relationship between the k_a's and true equilibrium binding constants (k^+, k^-) depends on the ratio between the rates of translocation of the loaded (κ) and unloaded (returning) carrier. In the simplest model, the symmetric carrier with identical loaded and unloaded translocation rates $k_a=k$ and $J_{ct}^{max}=$T.E. $\kappa/2$ where T.E. is the total amount of carrier in the membrane (Heinz, 1978).

By keeping one of the substrates constant while varying the other, the complexity of the analysis of Eq. (VII) can be greatly simplified. If, for example, the Cl concentration is kept constant, the equation can be rearranged to the standard Michaelis-Menten format

$$J_{ct}=\frac{V_{(Cl)}^{max}[K^+]}{K_{ct}^K+[K^+]}$$

with $K_{ct}^K=\alpha k_a^+(k_a^-+[Cl^-])/(\alpha k^-+Cl^-)$ and $V_{(Cl)}^{max}=J_{ct}^{max}[Cl^-]/(\alpha k^-+Cl^-)$. Following the Lineweaver-Burk analysis, if $J_{inf}=J_{ct}$, a double reciprocal plot of J_{inf} vs. $[K^+]$ for a given concentration of Cl^- will yield a straight line intercepting the $1/J_{inf}$ or y axis at $1/V_{Cl}^{max}$ and the $1/[K^+]$ or x axis at $1/K_{ct}^K$. When various concentrations of Cl^- are studied simultaneously a family of straight lines, crossing at a single point, is obtained (Segel, 1975). The common point of intersection has coordinates $-1/k^+$ on the x axis and $(1-\alpha)/J_{ct}^{max}$ on the y axis. If $\alpha=1$, the common intersection should occur on the x axis and $k^+=K_{ct}^K$. By plotting $1/(J_{inf})$ vs. $1/[Cl^-]$ for various fixed concentrations of K^+, the equivalent values for $V_{(K)}^{max}$ and K_{ct}^{Cl} can be obtained. Finally, double reciprocal plots of $V_{(S)}^{max}$ vs. (S), where $S=Cl^-$ or K^+, should intercept the $V_{(S)}^{max}$ axis at $1/J_{ct}^{max}$ and the S axis at $-1/\alpha k^-$ (for Cl^-) and $-1/\alpha k^+$ (for K^+).

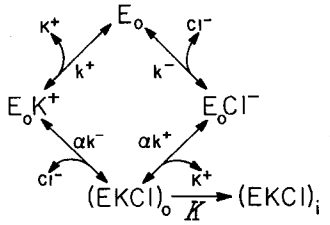


Fig. 8. Scheme for the random sequential model of KCl cotransport, k_1^- and k_2^+ are the equilibrium binding constants for Cl^- and K^+, respectively, in the absence of co-ion. α represents the extent to which binding of one ion can modify the affinity for the other. κ is the rate constant for the translocation of the complex across the membrane

Analysis of the Experimental Results by the Cotransport Model

The dependence of J_{inf} on substrates was analyzed according to the guidelines established in the preceding section by using the parameter $(\Delta dF/dt)_o$ within the limits of validity imposed in deriving Eq. (III). Double reciprocal plots of $(\Delta dF/dt)_o$ as a function of $[Cl^-]$ for concentrations of K^+ ranging from 1.5 to 20 mM are presented in Fig. 9*A*. The analogous set of results when K^+ was the variable for a series of fixed concentrations of Cl^- ranging between 4 and 20 mM are given in Fig. 9*B*. Only those experimental values for which $(\Delta dF/dt)_o \leqq 1/2\,(\Delta dF/dt)_o^{max}$ (i.e., $1/\Delta dF/dt)_o=2$) were taken into consideration for the manual construction of the slopes. In this way, Michaelis constants for K^+ and Cl^- equal to 15 ± 2 mM and 46 ± 5 mM, respectively, were obtained. Linear regression analysis using all experimental points smaller than 2/3 of $(\Delta dF/dt)^{max}$ yield essentially the same results.

Figure 10 shows a replot of the values of $1/V_{(Cl)}^{max}$ vs. $1/[Cl^-]$ and $1/V_{(K)}^{max}$ vs. $1/[K^+]$ from data given in Figs. 9*A* and 9*B*, respectively. According to theory, intersections on the x axis should yield $-1/\alpha k^-$ for the $1/V_{(Cl)}^{max}$ replot, and $-1/\alpha k^+$ for the $1/V_{(K)}^{max}$ replot. From these respective intercepts, we obtain values of $\alpha k^+ = 14$ mM and $\alpha k^- = 50$ mM. Since these values are essentially equal to the values calculated from Figs. 8*A* and 8*B* for K_{ct}^K and K_{ct}^{Cl}, it seems reasonable to conclude that $\alpha=1$. The $1/V_{(S)}^{max}$ lines for Cl^- and K^+ should have a common intersection on the y axis (equivalent to $1/J_{ct}^{max}$) and this prediction is closely followed. The calculated value for J_{inf} from Fig. 10 is about 8,

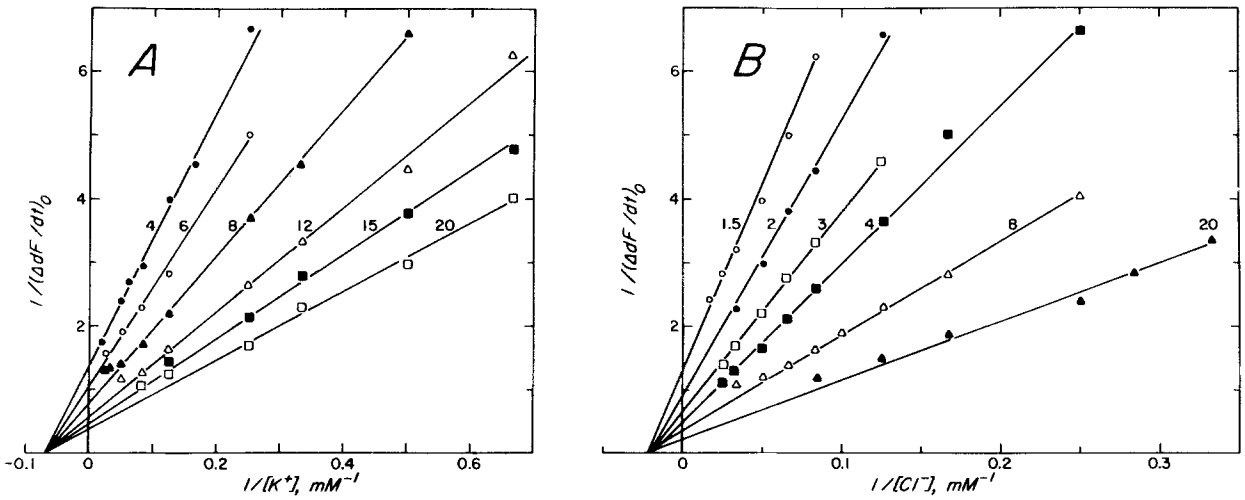


Fig. 9. Lineweaver Burk plots of $(\Delta dF/dt)_o$ vs. the concentration of Cl^- for various constant concentrations of K^+ (*A*) or vs. the concentration of K^+ for various constant concentrations of Cl^- (*B*). The lines were drawn taking into consideration only the points for which $(\Delta dF/dt)_o \leqq 1/2\,(\Delta dF/dt)_o$

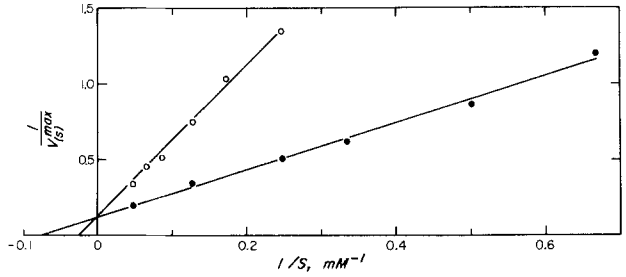


Fig. 10. Relationship between $1/V_{(S)}^{max}$ and $1/S$ for the data presented in Fig. 9. $S = Cl^-$ or K^+. The V_{Cl}^{max} values (*o*) were obtained from the *y*-axis intercepts for each $[Cl^-]$ of Fig. 9*A*; V_K^{max} values (●) were obtained from the *y*-axis intercepts for each $[K^+]$ of Fig. 9*B*

i.e., J_{ct}^{max} is 8 times larger than that of the experimentally observed maximum for $(\Delta dF/dt)_o$, which is taken to represent the maximum activity of the H^+/K^+ pump, J_p. Thus, the observed dependence of $(\Delta dF/dt)_o$ on the concentration of cation and anion can be explained by the combined action of a fast 1:1 KCl symport with defined affinities for K^+ and Cl^- and an ATP-driven H^+/K^+ pump.

Discussion

A comparative analysis of the functional properties of the stimulation-associated vesicles and gastric microsomes based on the known properties of the $(H^+ + K^+)$-ATPase enzyme leads to the conclusion that the differences between both vesicular types reside in the existence of an electroneutral KCl symport in the s.a. membrane. Further confirmation of this conclusion could be obtained by comparing the experimental kinetics with that predicted for cotransport systems. Knowledge of kinetic parameters could also be very valuable for the understanding of the oxyntic cell function.

In view of the technical difficulties presented by the study of kinetic features with isotopes, we devised an indirect method to measure the salt influx. The inward flux of KCl was measured by the resulting H^+ accumulation under the assumption that it will equal the salt flux when the latter is the rate-limiting step. In reaching Eq. (III) two assumptions were made: *a*) at the time the values for J_p are measured the backflux of KCl (J_{eff}) is much smaller than J_{inf}, and *b*) the system has reached (or is sufficiently close to) a steady state with respect to $[K^+]$. The experimental dH^+/dt values (and hence J_p) were obtained, not as absolute, but as relative values in the form of $(\Delta dF/dt)_o$, which were measured approximately 10 sec after initiation of the H^+ accumulation. The data obtained in this way were in remarkably good agreement with results calculated applying a theoretically derived equation for the influx of substrate through a 1:1 obligatory cotransport system, providing support for the validity of the experimental approach. Still it could be argued that coincidental or unforeseen compensating factors (e.g., artifacts arising from the indirect way in which J_p was estimated) might play a role in

bringing experimental results and theoretical predictions to confluence. Therefore, it is important to determine whether our final results are consistent with the initial assumptions that were made in order to achieve them. In other words, do the experimental results justify assumptions *a* and *b* described above?

Consider assumption *a* in a particular case. In the absence of H^+/K^+ exchange ($J_p=0$) and for $[KCl]_o=8$ mM, the cotransport system will be in equilibrium ($J_{eff}=J_{in}$) only when $[KCl]_i=8$ mM. When the pump is operating with $[KCl]_o=8$ mM, our experimental results (Fig. 7) show that $(\Delta dF/dt)<1/2(\Delta dF/dt)^{max}$, hence $[K^+]_i<K_p^K$. Since $K_p^K<0.5$ mM, J_{eff} can only be a small fraction of J_{in}^f. (This may no longer be the case when as a result of continued HCl accumulation $[Cl^-]_i$ is significantly larger than $[Cl^-]_o$ but our results were all obtained at low pH gradients.) The same argument may be applied to any of our experimental points for which the observed rate of fluorescence quenching is significantly smaller than the maximal rate, so that low values for $[K^+]_i$ can be ascertained. To test condition *b*, i.e., whether $[K^+]_i$ has approached a steady state at the time of measurement ($t\simeq 10$ sec), the equation for $[K^+]_i$ as a function of time has to be solved,

$$v_i\, d[K^+]_i/dt = J_{inf} - J_p = J_{inf} - \frac{J_p^{max}[K^+]_i}{K_p^K + [K^+]_i}$$

with $[K^+]_i=\chi K_p^K$, $N=J_p^{max}/J_{inf}$ and rearranging we obtain

$$\frac{1+\chi}{1+(1-N)\chi}\, d[K^+]_i = \frac{J_{inf}}{v_i}\, dt.$$

Integrating, we obtain

$$\frac{N}{(1-N)^2}\ln[1+(1-N)\chi] + \frac{\chi}{(1-N)} = \frac{J_{inf}}{v_i}\, t_o \quad \text{(VIII)}$$

where J_{inf}/v_i values have to be expressed in units of mM/min. J_{inf}/v_i values could be obtained from isotope flux measured as $C(\ln 2/t_{1/2})$ where C is the substrate concentration and $t_{1/2}$ is the half-time of equilibration. In the relative units of $(\Delta dF/dt)_o$, the rate of unidirectional flux (J_{inf}) for any concentration of K^+ and Cl^- can be calculated by introducing in Eq. (VII) the experimentally determined values for k_a^-, k_a^+, and J_{ct}^{max}. To transform this unidirectional flux from relative units into the absolute units of mM min^{-1}, J_{inf} values should be multiplied by the ratio of the values of J_{inf} in the absolute and relative units at any given concentration of K^+ and Cl^-. From Fig. 4*B* we have for $C=10$ mM, $t_{1/2}=0.167$ min leading to $J_{inf}=60$ mM/min. (This calculation should be taken only as a rough estimate since the short half-times of equilibration at 37 °C hindered an accurate determination of $t_{1/2}$.) The corresponding value of J_{inf} in relative units calculated from Eq. (VIII) using $k^-=46$ mM, $k^+=15$ mM, $\alpha=1$ and $J_{ct}^{max}=8$ is 0.56. Therefore, the constant for conversion into absolute units should be $60/0.56=107$ mM min^{-1}. Numerical solution of Eq. (VIII) using the calculated J_{inf}/v_i, and values of $K_p^k<0.5$ mM shows that for all experimentally relevant cases, i.e., $(\Delta dF/dt)_o<1/2(\Delta dF/dt)_o^{max}$, $[K^+]_i$ reaches a concentration of 95% of the steady-state value in less than 10 sec. More important, J_p reaches in this time a value which is within 1% of its "true" steady-state value. Because all our experimental measurements were started at least 10 sec after addition of the vesicles to the uptake medium, we feel that the steady-state assumption is justified. Thus the final results are able to sustain the original assumption made in developing Eq. (III), reinforcing the validity of the model proposed.

We can proceed now to offer an explanation for the differences in the profiles of acid accumulation between gastric microsomes and s.a. vesicles. In valinomycin-complemented gastric microsomes, acid accumulation is limited by the permeability of the anion: the more permeable the anion the larger the rate of KCl in flux, and subsequently the rate of H^+ uptake. In s.a. vesicles, when the concentration of a number of anions is high (e.g., 150 mM K^+ salt), $J_{inf}>J_p$. The ATPase then becomes saturated with K^+ and the rates of acid accumulation become identical for all these anions. As accumulation of HA acid increases, however, so does the rate of leak of H^+ and anion through dissipative pathways. When the rate of acid accumulation equals the rate of acid dissipation, the maximal gradient of pH has been reached. Hence, as may be seen in Fig. 1, the more permeable the anion the smaller the maximal pH achievable. In s.a. vesicles, as the concentration of the anion is decreased towards the point where J_{inf} is not greater than J_p (Fig. 3), the differences of interaction of the various anions with the symporter become apparent. When the concentration of anions is below 20 mM (and $K^+=100$ mM), the halogens are more efficient than nitrate in eliciting H^+-accumulation in

constrat to that which is seen in gastric microsomes.

The case of $CH_3SO_3^-$ is interesting in that the anion seems to be highly impermeable through conductive pathways. On the other hand, the anion seems to be slightly permeable through the electroneutral symport since it is able to elicit a reasonable rate of H^+ accumulation in s.a. vesicles when its concentration is high enough. In fact, at 250 mM $CH_3SO_3^-$ the rates of accumulation approach the maximal rates observed with the halogens. On the other hand, due to its limited conductive permeability it provides a poor dissipative pathway by H^+ and anion efflux. As a result, a larger ΔpH is achieved with $CH_3SO_3^-$ than with the more ionically permeable anions.

The effect of valinomycin on s.a. vesicles with preformed gradients is also consistent with these interpretations. Addition of the ionophore does not affect the H^+ gradient in the case of the halogens and nitrate. This suggests that KCl entry through the symport is more efficient than the valinomycin-mediated salt entry. Under the experimental conditions of Fig. 1 there is only a small gradient of K^+ across the membrane and valinomycin cannot seriously influence the rate of H^+ and anion dissipation. In the cases of $CH_3SO_3^-$ and isethionate, valinomycin cannot induce an increase in K^+-salt entry due to the impermeable character of the anions. On the other hand, since the concentration of K^+ inside the vesicles is probably low (a conclusion based on the observation that rates of uptake are significantly smaller than the maximal rates observable with other anions) addition of the ionophore may produce a large membrane potential which favors gradient dissipation. The accumulated protons which could not properly dissipative due to the low anion permeability can now flow down their electrochemical gradient (in to out); electroneutrality is preserved by K^+ which flows down its chemical gradient (out to in). A similar effect of valinomycin on maximal pH gradient, though less dramatic, is observed on gradients achieved in high K^+ and low Cl^- concentrations (*not shown*), but not on gradients achieved in high Cl^- and low K^+. This result can be expected from the preceding discussion.

Thus, the observed functional properties of the isolated apical membrane of the secreting oxyntic cell can be explained as the result of the combined action of the $(H^+ + K^+)$-ATPase pump which displays a high affinity for K^+ and the newly identified K^+ and anion electroneutral cotransport. This symport system displays a lack of significant interaction in the binding of anion and cation and relatively low discriminating power with respect to a number of anions. The calculated apparent Michaelis constants were about 15 and 46 mM for K^+ and Cl^-, respectively. The maximal velocity of KCl translocation as calculated from the cotransport expression is at least eight times larger than the maximal rate of K^+ recycling (i.e., H^+ pumping) by the pump. We cannot completely rule out the possibility that this larger ratio between the maximal rates of KCl translocation and ATP-driven H^+/K^+ exchange results from a physical segregation of KCl pathways and ATPase pumps occurring at the time of homogenization, so that H^+ uptake is being measured only in vesicles enriched in KCl symport activity. However, the fact that similar ratios were determined in at least five different membrane preparations from rabbit and large ratios have also been confirmed in preparations from piglet and rat stomachs (*unpublished observations*) tend to make the physical segregation hypothesis unlikely. Hence, the significance of these large ratios to the physiological function in intact tissue should be analyzed carefully. In a simplistic approach it might be expected that a considerable KCl secretion (the equivalent to KCl influx in the isolated vesicle), should occur in stimulated tissue. However, because the intracellular concentration of Cl is 20–30 mM, i.e., below the Michaelis constant for Cl^-, the actual KCl translocation will just exceed the maximal rates of H^+ pumping (*see* Fig. 3). In addition, the large Cl concentration present at the luminal face of the gastric epithelium might produce a significant back flux of KCl even at low K^+ concentrations, serving to prevent an increase in luminal K^+, and net K^+ secretion. Given the low value of K_p^K, the H^+/K^+ pump will still be able to produce maximal rates of acid secretion.

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