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# $Na^+$–$H^+$ exchange in gastric glands as measured with a cytoplasmic-trapped, fluorescent pH indicator

[amiloride/monensin/ouabain/pH regulation/2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein]

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**ABSTRACT** **We have used the pH-sensitive, fluorescent, cytoplasmic-trapped dye 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) to identify $Na^+$–$H^+$ exchange in gastric glands isolated from rabbit stomachs by high-pressure perfusion and collagenase digestion. The fluorescence of BCECF-loaded glands was calibrated in terms of cytosolic pH ($pH_c$) by permeabilizing the cell membranes and titrating the extracellular solution to different pH values. In one set of experiments in $Cl^-$-free solutions, glands were treated with 0.1 mM ouabain for 45 min to increase cellular cytosolic molar sodium ion concentration ($[Na^+]_c$) to high levels. Subsequent suspension of these cells in a $Na^+$-free Ringer's solution (to generate $[Na^+]_c > [Na^+]_o$) caused cells to acidify rapidly ($t_{1/2} \approx 60$ sec) from $pH_c \approx 7.15$ to $pH_c \approx 6.55$. Subsequent addition of 100 mM $Na^+$ or $Li^+$, but not $K^+$, caused cells rapidly to increase $pH_c$ ($t_{1/2} \approx 30$ sec) toward the control value. These changes of $pH_c$ were blocked when ouabain-treated glands had been preequilibrated for 10 min with 1 mM amiloride, and this block was overcome by adding 10 $\mu$M monensin (an ionophore that artificially exchanges $Na^+$ for $H^+$). In another set of experiments in $Cl^-$-containing Ringer's solution, glands were acid-loaded by treatment with 30 mM $NH_4Cl$ for 4 min, followed by washing the $NH_4Cl$ from the solutions. Under these conditions, $pH_c$ decreased from 7.02 to ≈6.5; subsequent alkalinization of cells back to control $pH_c$ was stimulated by $Na^+$ ($t_{1/2} \approx 60$ sec), but not $K^+$, and was inhibited by 1 mM amiloride. This amiloride block also was overcome by further addition of 10 $\mu$M monensin. We conclude that gastric glands contain a $Na^+$–$H^+$ exchanger that appears independent of $Cl^-$, not activated by $K^+$, and blocked by 1 mM amiloride. This exchanger is likely localized to the serosal membrane of gland cells. $Na^+$–$H^+$ exchange may play an important role in regulation of $pH_c$ in oxyntic and chief cells exposed to high luminal acidity, where back diffusion of $H^+$ into cells may occur at rapid rates.**

Studies of gastric physiology have traditionally centered around measurements of luminal acidity, and such measurements have provided a large body of information about the membrane mechanisms, regulatory aspects, and barrier function of the stomach. In contrast, there have been few studies of the cytosolic pH ($pH_c$) of gastric cells (1–4) and none concerned with how these cells might regulate their $pH_c$. Regulation of $pH_c$ is likely to be an important problem for all cells of the gastric mucosa. For example, during a meal, all cells of the gastric mucosa [surface (mucus-secreting), chief (enzyme-secreting), and oxyntic (acid-secreting)] will be exposed to a luminal contents with pH as low as 0.8 (e.g., see ref. 5). If the mucosal membranes of these cells have any $H^+$ permeability at all (e.g., ref. 6), then it is expected that there will be a back-leak of $H^+$ from the lumen into the cells. $H^+$ produced during metabolism also could contribute to cellular acidosis (7). Intracellular accumulation of $H^+$ in gastric cells generally is believed to be the *sine qua non* for the formation of ulcers, and if these cells have mechanisms for regulating $pH_c$, such regulation might contribute to the barrier function of the stomach.

The purpose of the present work was to investigate how gastric cells regulate their $pH_c$. We have utilized the pH-sensitive dye 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF), which has been shown to be a useful probe for continuous measurements of cytoplasmic pH in a variety of cells (8, 9). BCECF has a $pK_a$ of 6.98, exhibits a practically linear relationship between pH and fluorescence (at least between pH 6.4 and 7.4), and is relatively membrane impermeant because it contains four carboxyl groups. Addition of acetoxymethyl ester groups to these carboxyls yields BCECF/AM, which is membrane permeable and easily enters cells. Intracellular esterases cleave the BCECF/AM back to membrane-impermeant BCECF, and continuous measurements of the fluorescence from the trapped dye are then made on gastric glands suspended in a spectrofluorometer. Calibration of the fluorescent signal is performed at the end of each experimental run to obtain quantitative measurements of $pH_c$.

Since much recent work in other cell types has implicated $Na^+$–$H^+$ exchange as a common mechanism for regulating $pH_c$, we have performed experiments designed to test for the presence of this carrier in isolated rabbit gastric glands. One basic protocol was to monitor $pH_c$ during experimental conditions in which the molar $Na^+$ concentration gradient across the cell membranes was oriented either outward ($[Na^+]_c > [Na^+]_o$) or inward ($[Na^+]_o > [Na^+]_c$). The concentration gradient was established by treating the glands with ouabain in solutions with different $[Na^+]$. The expectation was that, if a $Na^+$–$H^+$ exchanger existed, when the $Na^+$ gradient was oriented outward, $Na^+$ would move out of the cell in exchange for $H^+$ and the cells would acidify, and that reversing the $Na^+$ gradient would reverse this acidification. We utilized $Cl^-$-free solutions for these initial experiments for two reasons. First, $Cl^-$-free solutions reduce the amount of acid contained in intracellular tubulovesicles of oxyntic cells (10), and we hoped this $Cl^-$-free treatment would reduce any potential problems caused by BCECF which leaked into these acidic vesicles. Second, we hoped to eliminate any contribution from a $Cl^-$–$OH^-$ ($HCO_3^-$) exchanger, which also may be present. A second protocol was to acidify cells by using the ammonium-loading technique popularized by Boron and his collaborator (e.g., see ref. 7) and then to study the $Na^+$ dependence of $pH_c$ regulation. We utilized $Cl^-$-containing Ringer's solution for these experiments because the presence or absence of $Cl^-$ did not appear to alter

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Abbreviations: $pH_c$ and $pH_o$, cytosolic and outside pHs; $[Na^+]_c$ and $[Na^+]_o$, cytosolic and outside molar $Na^+$ concentrations; BCECF, 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein; BCECF/AM, acetoxymethyl ester of BCECF; AP, aminopyrine.

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the basic findings regarding the $Na^+$ dependence of $pH_c$ regulation in the glands. Our data indicate that isolated gastric glands do indeed contain a $Na^+$–$H^+$ exchanger, which is inhibited by amiloride.

## MATERIALS AND METHODS

**Chemicals and Solutions.** Monensin, Hepes, collagenase, choline chloride, and choline bicarbonate were from Sigma; digitonin, from Nutritional Biochemicals; methanesulfonic acid, from Aldrich; and amiloride, from Merck Sharp & Dohme. BCECF/AM was prepared by one of us (R.Y.T.) following the synthesis as described (8). Stock solutions of BCECF/AM (10 mM) and digitonin (10 mg/ml), both in dimethyl sulfoxide, and monensin (10 mM) in dimethylformamide/ethanol, 3:1 (vol/vol), were stored at −20°C. Stock solutions of $NaCH_3SO_3$ and choline $CH_3SO_3$ (both at 1 M, pH 7.0) were prepared by mixing 2 M $CH_3SO_3H$ with equimolar concentrations of NaOH and choline bicarbonate, respectively, and were stored at 2°C. Choline $CH_3SO_3$ was bubbled with 100% $N_2$ for 12 hr to remove residual bicarbonate. All reagents were of the highest chemical grade. The various Ringer's solutions used in this study were as follows: NaCl-Ringer's solution was 150 mM NaCl/2.5 mM $K_2HPO_4$/1.0 mM $CaSO_4$/1.0 mM $MgSO_4$/11.1 mM glucose/20 mM Hepes; for $NaCH_3SO_3$-Ringer's solution, we replaced NaCl with $NaCH_3SO_3$; choline Cl-Ringer's solution was 150 mM choline Cl/2.5 mM $K_2HPO_4$/1.0 mM $CaSO_4$/1.0 mM $MgSO_4$/20 mM Hepes; for choline $CH_3SO_3$-Ringer's solution, the $Cl^-$ was replaced by $CH_3SO_3^-$. All solutions were adjusted to pH 7.38 ± 0.02 at 24°C.

**Preparation of Gastric Glands.** Rabbit gastric glands were prepared as described by Berglindh and his colleagues (10–12) using abdominal vascular, high-pressure perfusion and collagenase digestion. Briefly, the animals were first anesthetized with a medium-range cocktail containing ketamine, acepromazine, and xylazine and then placed in a surgical plane using sodium pentobarbital. The abdomen was quickly opened, and the descending aorta was cannulated and perfused with 150 mM NaCl/3 mM $K_2HPO_4$/0.6 mM $NaH_2PO_4$. The animal then was killed with an overdose of sodium pentobarbital. The stomach was removed from the animal, scraped from the underlying connective tissue, and minced with scissors. The minced tissue was then digested (with 2 mg of type 1A collagenase per ml) in Eagle's minimal essential medium containing Hanks' salts, L-glutamine, nonessential amino acids, 1 mg of albumin per ml, and 20 mM Hepes (pH 7.4) under 100% $O_2$ at 37°C until glands were formed (usually 45 min).

**Measurement of $pH_c$ Using BCECF.** Isolated glands were washed several times in the same digestion medium without collagenase, and then 2–4 μM BCECF/AM was added to the suspension. Glands were incubated (under 100% $O_2$) with BCECF for ≈1 hr at 37°C. The impermeant BCECF is generated *in situ* by the action of cytoplasmic esterases (8); ≈15 μM BCECF was trapped in the glands during this process.

After the loading procedure, glands were washed four times over a 45-min period, with frequent resuspensions at room temperature in a specific incubation medium before being resuspended in the final test medium. This washing procedure removed almost all of the extracellular dye, and only the cytoplasmic-trapped dye remained. The dye appeared under fluorescence microscopy to be evenly distributed in the cytoplasmic space of all cells in the glands. BCECF-loaded glands appeared in all ways to behave "normally." Control and BCECF-loaded glands both excluded erythrosin B to the same extent—90%. And both sets of glands exhibited the same ability to secrete $H^+$ as judged by the accumulation of the weak base aminopyrine (AP; $^{14}C$ label; see ref. 12 for a description of the method): control glands had AP ratios ($[AP]_{glands}/[AP]_{outside}$) of 23.4 ± 0.8 ($n$ = 4) in the resting state and 251 ± 12 ($n$ = 4) after stimulation with the potent secretagogue dibutyryl cAMP (1 mM); BCECF-loaded glands had AP ratios of 26.2 ± 1.5 ($n$ = 4) in the resting state and 243 ± 8 ($n$ = 4) during stimulation with dibutyryl cAMP.

BCECF also appears to behave "normally" when it is incorporated into the cells because the emission spectrum of the dye in free solution and when incorporated into glands exhibited emission maxima at 526 nm. Continuous fluorescence (emission at 526 nm; excitation at 490 nm, slits of 5 × 3 nm) of dye-loaded glands (at 5% packed cell volume) was monitored in the ratio mode by gently suspending glands in a thermostatically controlled (24 ± 2°C) cuvet in a Perkin–Elmer MPF-44A spectrofluorometer equipped with a magnetic stirrer. Under these conditions, the leakage rate of the dye from glands, estimated by initial and final dye in the supernatant, changed by <3% of the total fluorescent signal over a 10-min period—the normal duration of most of the experimental protocols. This total background fluorescence rarely exceeded 10% of the total fluorescent signal. Thus, BCECF leaks only slowly from rabbit gastric glands during the various procedures used here.

The fluorescence signal was calibrated to yield $pH_c$ by the following protocol. At the end of an experimental procedure, $pH_c$ and the outside pH ($pH_o$) were equilibrated by permeabilizing the cells with 50 μM digitonin or 0.03% Triton X-100 (added to the incubation solution). Then the solution was titrated with either 1 M HCl or 1 M NaOH over the range of fluorescence values obtained during the experiment. By measuring the solution pH after each addition of acid, a calibration curve of fluorescence vs. pH was constructed for each experimental sample. BCECF exhibited a nearly linear relationship between fluorescence and pH, at least over the pH range 6.4–7.4. We constructed similar calibration curves by using a variety of permeabilizing agents (nigericin in the presence of high $[K^+]$ and monensin for ouabain-treated cells in high $[Na^+]$), and they all yield $pH_c$ values that agree within 0.05–0.15 pH units. In general, the $pH_c$ values reported here are ≈0.1 pH unit underestimated compared with the $pH_c$ values obtained with monensin. Since the main purpose of using BCECF was to monitor changes in $pH_c$, the values reported here were not corrected to those obtained with either nigericin or monensin. We will report the details of these calibration schemes in more detail in a subsequent paper.

Interference due to autofluorescence and light scatter of unloaded glands represented <3% of the total signal of BCECF-loaded glands. During the various protocols, interference changed by <1% at the instrument settings used in this study; therefore, these changes were ignored.

We tested whether any dye had penetrated the mitochondria by using valinomycin and rotenone, two agents that are known to alter the pH gradient across the inner mitochondrial membrane. Valinomycin should cause pH of the inner mitochondrial space to become more alkaline, whereas rotenone should cause this space to become more acidic. Previous experiments have shown that fluorescein, but not carboxyfluorescein, monitors these changes of mitochondrial pH quite faithfully both in isolated mitochondria and in Ehrlich ascites tumor cells (13). In the present experiments, neither 10 μM valinomycin ($n$ = 4) nor 10 μM rotenone ($n$ = 2) had any detectable effect on the fluorescence of BCECF-loaded glands. We conclude that the dye is excluded from the mitochondria. Other experiments to be reported later indicate that BCECF is likely not responding to acidic compartments (e.g., lysosomes or tubulovesicles with pH < 5.0) of glandular cells.

## RESULTS

**Effects of Ouabain and Amiloride on $pH_c$ in $NaCH_3SO_3$- and Choline $CH_3SO_3$-Ringer's Solutions.** In direct comparisons of resting gastric glands in Ringer's solutions, AP ratios in NaCl-Ringer's solution averaged 8.4 ± 0.1 ($n$ = 4), whereas in $NaCH_3SO_3$-Ringer's solution, AP ratios averaged half this value, 4.2 ± 0.1 ($n$ = 4). It appears, then, that $NaCH_3SO_3$-Ringer's solution does reduce $H^+$ accumulation in acidic spaces of resting glands (see ref. 10).

When BCECF-loaded glands were incubated for 1 hr in $NaCH_3SO_3$-Ringer's solution and then suspended in a cuvet in the same solution, the fluorescence trace remained stable for many minutes, and $pH_c$ was always around 7.15. Average $pH_c$ for glands incubated in $NaCH_3SO_3$-Ringer's solution was 7.15 ± 0.05 ($n$ = 11). A similar response was found for glands incubated in $NaCH_3SO_3$-Ringer's solution containing 0.1 mM ouabain for 45 min (Fig. 1, trace A). [It has been demonstrated previously that this length of ouabain treatment is sufficient to cause the cells to lose nearly all of their $K^+$ and gain $Na^+$ to high values (up to 125 mM; see ref. 11).] Average $pH_c$ for such ouabain-treated glands in $NaCH_3SO_3$-Ringer's solution was 7.18 ± 0.06 ($n$ = 11). In contrast, when ouabain-treated glands in $NaCH_3SO_3$-Ringer's solution were suspended in choline $CH_3SO_3$-Ringer's solution, the cells acidified at a rapid rate to pH 6.55 (Fig. 1, trace B). When 100 mM $NaCH_3SO_3$ was subsequently added to the solution (arrow with Fig. 1, trace B), the pH of the cells increased back toward the control $pH_c$. When 100 mM $KCH_3SO_3$ was added instead of $NaCH_3SO_3$, the $pH_c$ did not change—it remained acidic ($n$ = 6; not shown). $Li^+$ is the only ion we have tested that was able to substitute for $Na^+$ to cause realkalinization of $pH_c$.

It should be noted that, for the protocol shown in Fig. 1, trace B, the $Na^+$-dependent realkalinization of ouabain-treated glands was accomplished by adding 100 mM $NaCH_3SO_3$ on top of the choline $CH_3SO_3$-Ringer's solution in which the glands were suspended. Thus, the glands were being exposed to hypertonic solutions. To test whether the hypertonicity may have stimulated a $Na^+$–$H^+$ exchanger that normally is inactive, we did the following experiment. Ouabain-treated glands were incubated in $NaCH_3SO_3$-Ringer's solution, washed once in choline $CH_3SO_3$-Ringer's solution, and finally suspended in $NaCH_3SO_3$-Ringer's solution. In this protocol, the glands were exposed only to isosmotic solutions, and the $pH_c$ of these glands started out initially at pH 6.6 and immediately increased back toward control $pH_c$ levels (Fig. 1, trace C).

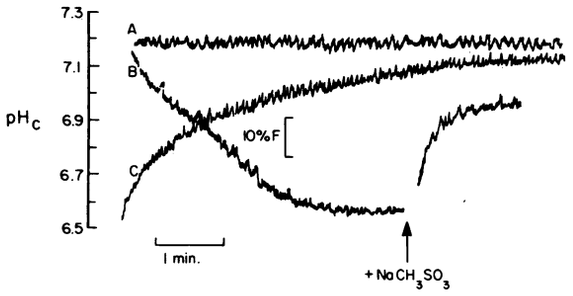


FIG. 1. Effects of $Na^+$ and $Na^+$-free treatment on $pH_c$ in ouabain-treated glands in $Cl^-$-free Ringer's solution. Glands were incubated in $NaCH_3SO_3$-Ringer's solution containing 0.1 mM ouabain for 45 min. These glands were then washed once in the same or another different solution before suspension in the final test solution: glands were resuspended in $NaCH_3SO_3$-Ringer's solution containing ouabain, and the $pH_c$ remained constant at 7.15 (trace A); glands were resuspended in choline $CH_3SO_3$-Ringer's solution containing ouabain, and the $pH_c$ decreased from 7.15 to 6.55 ($t_{1/2} \approx 60$ sec), and this acidification was reversed when 100 mM $NaCH_3SO_3$ was added back (arrow) to the solution (trace B); and glands were first washed in choline $CH_3SO_3$-Ringer's solution containing ouabain for 5 min and then resuspended at the beginning of the trace in $NaCH_3SO_3$-Ringer's solution, and cells became alkaline at rates very similar (but not identical) to those observed in trace B, where the solution was hypertonic (trace C). All traces represent one of six identical experiments.

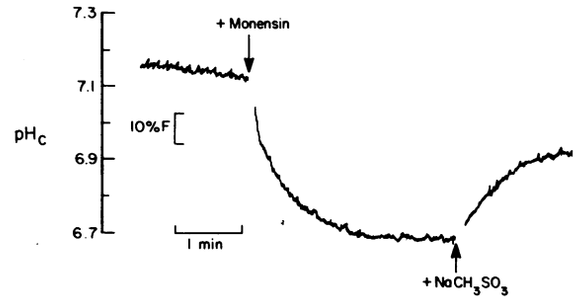


FIG. 2. Effects of amiloride and monensin on $pH_c$ in ouabain-treated glands. Glands were treated with 0.1 mM ouabain in $NaCH_3SO_3$-Ringer's solution for 45 min. During the last 10 min of incubation, 1 mM amiloride was included also. These ouabain- and amiloride-treated glands were then suspended in choline $CH_3SO_3$-Ringer's solution containing ouabain, and the normal acidification process was blocked. Monensin at 10 μM and later $NaCH_3SO_3$ at 100 mM were added as shown. The trace is representative of six similar experiments.

$Na^+$–$H^+$ exchange is normally inhibited by high concentrations of amiloride. We tested the effects of 1 mM amiloride by using the protocol shown in Fig. 2. Glands were treated for 45 min with ouabain in $NaCH_3SO_3$-Ringer's solution as previously described and then were treated with amiloride for an additional 10 min in the same solution before resuspending in choline $CH_3SO_3$-Ringer's solution. In contrast to the control glands (i.e., no amiloride; Fig. 1, trace B), amiloride largely prevented the acidification associated with suspension in choline $CH_3SO_3$-Ringer's solution. This amiloride block was overcome when 10 μM monensin (an ionophore that artificially exchanges $Na^+$ for $H^+$) was added to the suspending medium (Fig. 2, first arrow): cells acidified to pH 6.6. Adding 100 mM $NaCH_3SO_3$ back to the solution (Fig. 2, second arrow) reversed the monensin-induced acidification.

**Ammonium-Treated Glands.** We also were interested to test for the presence of $Na^+$–$H^+$ exchange under rather more physiological conditions; therefore, we performed a series of experiments in NaCl-Ringer's solutions. For these control glands, average $pH_c$ = 7.02 ± 0.06 ($n$ = 21). When glands were incubated first in NaCl-Ringer's solution and then diluted into choline Cl-Ringer's solution, the $pH_c$ decreased slowly and then returned back to the control $pH_c$ when 100 mM NaCl was added back to the solution (Fig. 3). More dramatic decrease of $pH_c$ occurred when using an $NH_4^+$-loading procedure as shown in Fig. 4. Glands were incubated in NaCl-Ringer's solution, and then 30 mM $NH_4Cl$

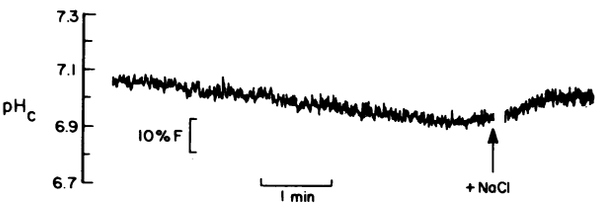


FIG. 3. Effects of $Na^+$-free treatment on control glands incubated in $Cl^-$-containing Ringer's solution. Glands were incubated in NaCl-Ringer's solution and suspended in choline Cl-Ringer's solution; 100 mM NaCl was added as shown. The trace is representative of six similar experiments.

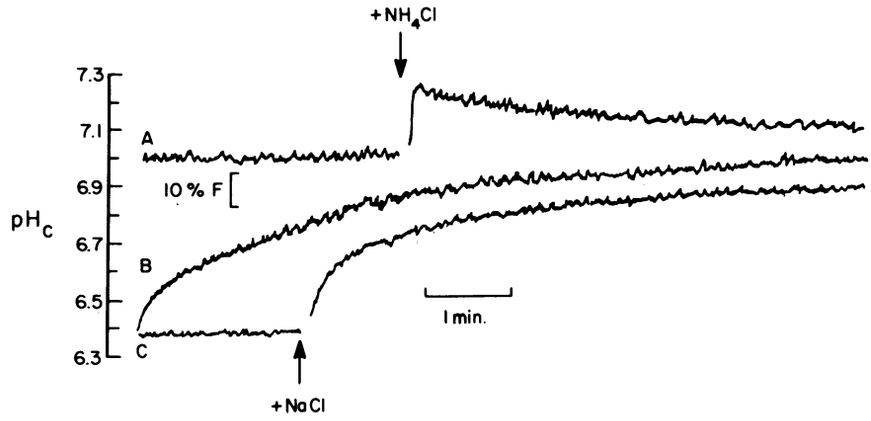


FIG. 4. Effects of $NH_4Cl$ on $pH_c$ of glands in NaCl-Ringer's solution and choline Cl-Ringer's solution. All traces represent one of six similar experiments. Traces: A, glands were suspended in NaCl-Ringer's solution, 30 mM $NH_4Cl$ was added (arrow), and the $pH_c$ increased from 7.0 to 7.25 and then "relaxed" back toward the control $pH_c$; B, glands were suspended in NaCl-Ringer's solution containing 30 mM $NH_4Cl$ for 4 min, washed once in choline Cl-Ringer's solution, and then suspended in NaCl-Ringer's solution at the beginning of the trace, which shows glandular cells regulating $pH_c$ back to control levels; C, glands were incubated in NaCl-Ringer's solution containing 30 mM $NH_4Cl$ for 4 min, washed once in choline Cl-Ringer's solution, and then suspended in choline Cl-Ringer's solution. Trace C shows that gland cells did not regulate $pH_c$ until 100 mM NaCl was added to the suspension (arrow).

was added (arrow with Fig. 4, trace A). This treatment caused $pH_c$ first to increase and then to "relax" back toward baseline, similar to the effects observed in other cell types (e.g., see ref. 7). If glands were treated with 30 mM $NH_4Cl$ for 4 min and then washed quickly in choline Cl-Ringer's solution before resuspending in NaCl-Ringer's solution, glands were initially rather acidic, but they rapidly regulated $pH_c$ back to control levels (Fig. 4, trace B). The regulatory phase was prevented if the $NH_4^+$ treatment was followed by washing and suspending glands in choline Cl-Ringer's solution (Fig. 4, trace C). Regulation of $pH_c$ occurred only after addition of NaCl to the choline Cl-Ringer's solution. $Na^+$-dependent regulation of $pH_c$ also was largely prevented by 1 mM amiloride, and the amiloride block was overcome when 10 $\mu$M monensin was added to the solution (Fig. 5).

## DISCUSSION

**Benefits of BCECF for Measuring $pH_c$.** pH-sensitive dyes in general are much more useful than such techniques as measuring the cellular–extracellular distribution of weak acids or weak bases because the time resolution of dyes is much faster (e.g., equilibration of the weak acid 5,5-dimethyl-2,4-oxazolildinedione takes about 60 min in frog gastric mucosa; see ref. 4). BCECF is a particularly useful fluorescent probe for a variety of reasons. First, its $pK_a$ (7.00) and linear range of response (pH 6.4–7.4) are close to the values observed in gastric glands during the course of our studies. Also, the fact that its emission spectrum is identical in suspended glands and in free solution indicates that the dye has not been altered to any significant extent by incorporation into gland cells. Second, BCECF appears to be a cytoplasmic-trapped dye that therefore monitors mainly cytoplasmic pH. This is in direct contrast to such dyes as bromothymol blue (2), which monitors the whole cellular compartment including mitochondria. Because mitochondria have an internal compartment that is more alkaline than the cytoplasm and because these organelles take up a sizeable fraction of the cytoplasm of oxyntic cells (e.g., see ref. 5), these latter dyes yield values of $pH_c$ that are likely to be overestimates of the true values. The last benefits of BCECF are that it is easy to use and calibrate and it leaks from gastric glands only very slowly, at least at room temperature.

**The $Na^+$–$H^+$ Exchanger Is Reversible and Inhibited by Amiloride.** When an in > out gradient of $[Na^+]$ was established, cells acidified (Fig. 1, trace B). The rate of acidification was larger for ouabain-treated glands (Fig. 1, trace B) than for control glands (Fig. 3), presumably because the in > out gradient of $[Na^+]$ was larger in the former than in the latter. After the $Na^+$-free treatment caused the cells to acidify, adding back 100 mM $Na^+$ (or $Li^+$, but not $K^+$) caused the $pH_c$ of the glands to increase back toward control values (Fig. 1, traces B and C). These data plus the fact that amiloride largely blocked the $Na^+$-free-treatment-induced acidification (and this amiloride block was in turn overcome with the artificial $Na^+$–$H^+$ ionophore monensin; Fig. 2) indicate that a $Na^+$–$H^+$ exchanger is present and, further, that it can operate reversibly depending on the gradient of $[Na^+]$. In this sense, the $Na^+$–$H^+$ exchanger of gastric glands behaves similarly to that found in renal brush border membranes (14).

We believe that the ouabain and $NaCH_3SO_3$-Ringer's solution were not artificially inducing a $Na^+$–$H^+$ exchanger that is not normally present because we obtained essentially the same results when the cells were acidified in control NaCl-Ringer's solution by the $NH_4^+$-loading technique. Thus, when the $NH_4Cl$ was removed from the bathing solu-

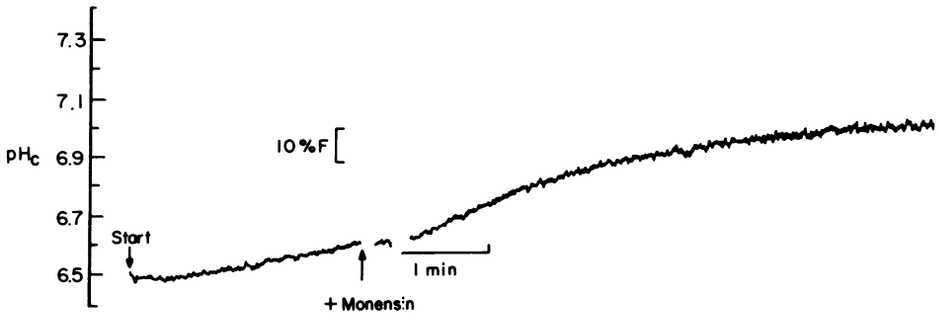


FIG. 5. Effects of amiloride on $pH_c$ regulation. Glands were first incubated in NaCl Ringer's solution containing 30 mM $NH_4Cl$ for 4 min. The glands were then washed once in choline Cl-Ringer's solution containing 1 mM amiloride before resuspension in NaCl-Ringer's solution. Glandular cells did not regulate $pH_c$ back toward control $pH_c$ until 10 $\mu$M monensin was added to the suspension (arrow). The trace is representative of six similar experiments.

tions, the cells became acidic, but they regulated the $pH_c$ back to control levels in the presence of $Na^+$ (Fig. 4, traces B and C) but not in its absence (Fig. 4, trace C) or during treatment with amiloride (Fig. 5).

It was noted that addition of monensin to amiloride-treated, $NH_4^+$-loaded cells caused the $pH_c$ to increase, but the rate of alkalinization was slow (e.g., compared to the effect of monensin in ouabain- and amiloride-treated glands, Fig. 2). The explanation for this difference may be that monensin exhibits a selectivity of $Na^+$:$K^+$ of only 6:1. Thus, in $NH_4^+$-treated glands where $[K^+]_c \approx 0.125$ M, $[K^+]_o = 0.5$ M, $[Na^+]_o = 0.150$ M, and $[Na^+]_c \approx 0.04$ M (11), monensin will induce both $Na^+$–$H^+$ and $K^+$–$H^+$ exchange, and the exchanges will occur in opposite directions, with $Na^+$–$H^+$ exchange tending to dominate. In contrast, in ouabain-treated glands suspended in $Na^+$-free Ringer's solution, it is expected that $[Na^+]_c/[Na^+]_o > 100$, $[K^+]_c \approx 0.037$ M, and $[K^+]_o = 0.005$ M (11). In this situation, monensin will induce mainly $Na^+$–$H^+$ exchange. Thus, monensin induces a large effect to overcome the amiloride block in ouabain-treated glands (Fig. 2) but not in $NH_4$-treated glands (Fig. 5).

**$Na^+$–$H^+$ Exchange in the Stomach: Which Cell Type? Which Membrane? Physiological Role?** It has been demonstrated that both newborn and adult rabbit stomachs actively absorb $Na^+$ (from mucosa to serosa) in an electrogenic fashion—i.e., changes in net $Na^+$ transport are mirrored by equivalent changes of short-circuit current (15). Low concentrations (1 $\mu$M) of amiloride added to the mucosal (but not the serosal) solution inhibit this $Na^+$ transport (16), and this inhibition does not alter rates of $H^+$ secretion into the stomach lumen (17). Therefore, it appears that inhibition of $Na^+$ movements across the mucosal membranes of gastric cells does not alter rates of $H^+$ movements at the same membrane. Also, all $Na^+$–$H^+$ exchangers that have so far been examined appear to be neutral, whereas movement of $Na^+$ across the mucosal membrane of gastric cells is electrogenic (e.g., see ref. 5). Finally, the ability of dibutyryl cAMP-stimulated gastric glands to accumulate the weak base AP 10-fold above levels of nonstimulated glands argues for a diffusion barrier between the site of secretagogue-stimulated secretion (at the apical membrane of oxyntic cells) and the bulk phase of the medium, which bathes the serosal membranes. Thus, we tentatively conclude that the $Na^+$–$H^+$ exchanger we have demonstrated here resides at the serosal membrane of gastric cells. As for the specific cell type involved, we cannot yet determine whether it exists in oxyntic cells, chief cells, or both. Experiments on purified populations of these two cell types will be required to settle this question.

An obvious role for $Na^+$–$H^+$ exchange at the serosal membranes of cells in gastric glands is the maintenance of a neutral $pH_c$. The ultimate energy for driving this system is derived from the serosal-to-cell $Na^+$ gradient, which in turn is maintained by the $Na^+$,$K^+$-ATPase located at the serosal membrane of these cells (see refs. 18 and 19). In this regard we note that glands in $Cl^-$-free Ringer's solution had a $pH_c \approx 7.15$, whether in the control (where $[Na^+]_o/[Na^+]_c > 3.5$) or the ouabain-treated state (where $[Na^+]_o/[Na^+]_c \approx 1$). The explanation for this apparent contradiction (i.e., equal $pH_c$ in the face of markedly different transmembrane gradients of $[Na^+]$) may be as follows. In control glands in $Cl^-$-free Ringer's solution, the cell potential across the serosal membrane is $-60$ mV (cell negative; see ref. 19), and the $pH_c$ would tend, from simple thermodynamic considerations, to be acidic. Specifically, with $pH_o = 7.4$, equilibrium $pH_c = 6.4$. However, the activity of the $Na^+$–$H^+$ exchanger keeps $pH_c \approx 7.15$. In ouabain-treated glands, the cell membrane potential is likely to be close to 0 mV, so even though there is little or no transmembrane gradient of $[Na^+]$, $pH_c \approx pH_o$ because $H^+$ is approximately at equilibrium. Thus, it is merely fortuitous that $pH_c$ is $\approx 7.15$ for both control and ouabain-treated glands in $Cl^-$-free solutions.

*In vivo* the activity of the $Na^+$–$H^+$ exchanger could be especially important for chief cells during conditions in which the oxyntic cells are active, and the glandular lumen is acidic. In this case, $H^+$ might leak into the cells and then be extruded into the serosal compartment and blood capillaries by the action of the $Na^+$–$H^+$ exchanger, which in turn is established by the operation of the $Na^+$,$K^+$-ATPase. Another use for the $Na^+$–$H^+$ exchanger may be as a means for generating so-called nonacidic $Cl^-$ transport. For example, if both $Na^+$–$H^+$ and $Cl^-$–$HCO_3^-$ exchangers operate in parallel at the serosal membrane of gastric cells, then these cells could accumulate $Cl^-$ to a level above electrochemical equilibrium [as has been observed (20) for surface cells], driven by the $OH^-/HCO_3^-$ gradient, which in turn is established by the operation of the $Na^+$–$H^+$ exchanger. If $Cl^-$ conductance channels exist at the mucosal membranes of glandular cells, then $Cl^-$ would leak out passively, generating a net, electrogenic $Cl^-$ secretion. For detailed discussions of such a system, we refer the interested readers to two recent reviews (18, 19).

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