Intracellular pH and Na Fluxes in Barnacle Muscle with Evidence for Reversal of the Ionic Mechanism of Intracellular pH Regulation

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ABSTRACT The ion transport mechanism that regulates intracellular pH (pHi) in giant barnacle muscle fibers was studied by measuring pHi and unidirectional Na⁺ fluxes in internally dialyzed fibers. The overall process normally results in a net acid extrusion from the cell, presumably by a membrane transport mechanism that exchanges external Na⁺ and HCO₃⁻ for internal Cl⁻ and possibly H⁺. However, we found that net transport can be reversed either by lowering [HCO₃⁻]₀ and pHᵢ₀ or by reducing [Na⁺]ᵢ₀. This reversal (acid uptake) required external Cl⁻, was stimulated by raising [Na⁺]ᵢ₀, and was blocked by SITS. When the transporter was operating in the net forward direction (acid extrusion), we found a unidirectional Na⁺ influx of ~60 pmol·cm⁻²·s⁻¹, which required external HCO₃⁻ and internal Cl⁻ and was stimulated by cyclic AMP and blocked by SITS or DIDS. These properties of the Na⁺ influx are all shared with the net acid extrusion process. We also found that under conditions of net forward transport, the pH-regulating system mediated a unidirectional Na⁺ efflux, which was significantly smaller than the simultaneous Na⁺ influx. These data are consistent with a reversible transport mechanism which, even when operating in the net forward direction, mediates a small amount of reversed transport. We also found that the ouabain-sensitive Na⁺ efflux was sharply inhibited by acidic pHi, being totally absent at pHi values below ~6.8.

INTRODUCTION

A central role for Na⁺ in the regulation of intracellular pH (pHi) is now well established. Squid giant axons, snail neurons, and the muscle fibers of the giant barnacle all regulate their pHi by a Na⁺-dependent acid extrusion process.¹ The ionic mechanism of acid extrusion is believed

¹ We define "acid extrusion" as the sum of the active uptake of base by the cell and/or removal of acid from the cell.

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equivalent to that illustrated in Fig. 1A, the uptake of Na\(^+\) and HCO\(_3^-\) in exchange for Cl\(^-\) and possibly H\(^+\) (Thomas, 1977). Acid extrusion in barnacle muscle is known to have an absolute dependence upon both external Na\(^+\) (Boron et al., 1981) and external HCO\(_3^-\) (Boron et al., 1977, 1981), to be inhibited by the stilbene derivative SITS (4-acetamido-4'-isothiocyanostilbene-2',2'-disulfonic acid) (Boron, 1977), and to be stimulated by cyclic AMP (Boron et al., 1978). Furthermore, the rate of acid extrusion is inversely related to pH\(_i\) (Boron et al., 1978, 1979). In addition to the implied HCO\(_3^-\) and possibly H\(^+\) fluxes, the pH\(_i\)-regulating system is believed to mediate a component of both unidirectional Cl\(^-\) influx and Cl\(^-\) efflux. The evidence for this latter statement is that unidirectional Cl\(^-\) fluxes are greatly stimulated by an acidic pH\(_i\) and these pH\(_i\)-stimulated Cl\(^-\) fluxes are further enhanced by cAMP and inhibited by SITS (Boron et al., 1978). However, despite the observation that Na\(^+\) is required for acid extrusion and that net Na\(^+\) uptake can be predicted from the model of Fig. 1A, there have been no studies of Na\(^+\) fluxes mediated by the barnacle's pH\(_i\)-regulating system.

The extra Cl\(^-\) efflux occasioned by an acidic pH\(_i\) could be predicted on the basis of the model of Fig. 1A. However, the extra Cl\(^-\) influx is unexpected and suggests that the pH\(_i\)-regulating system may also mediate a backleak of one or more ions across the membrane. One possible mechanism of such a backleak is that, even when transport proceeds in the direction of net acid extrusion (Fig. 1A), a fraction of the transporters may operate in reverse (Fig. 1B), mediating the unidirectional influx of Cl\(^-\) plus H\(^+\) and the unidirectional efflux of Na\(^+\) plus HCO\(_3^-\). If such microscopic reversibility of the transporter is possible under conditions of net forward transport, one might expect that net reversal of the transport could be produced by appropriately altering the gradients of one or more of the transported ions. The first experimental support of the net reversal concept came from Boron's (1977) observation that when barnacle muscle was exposed to CO\(_2\) at very low external pH (pH\(_o\)) and [HCO\(_3^-\)], the pH\(_i\) not only failed to recover from the CO\(_2\)-induced intracellular acid load, but continuously declined. The net uptake of acid that produces this intracellular acidification has two characteristics consistent with the model of Fig. 1B: it depends on HCO\(_3^-\) and it is blocked by SITS (Boron et al., 1979). Further evidence for a net reversal of the pH\(_i\)-regulating system was provided by Keifer's (1979) observation that the pH\(_i\) in a dialyzed barnacle muscle decreased when [Na\(^+\)]\(_i\) was raised and increased when [Na\(^+\)]\(_o\) was lowered. Finally, using snail neurons, Thomas (1980) showed that the fall in pH\(_i\) that accompanies the reduction of pH\(_o\) is blocked either by removal of external Cl\(^-\) or by application of SITS.

In the present study, we used pH-sensitive microelectrodes to examine the purported pH\(_i\)-regulating system under conditions that should favor net reversal of transport. Our results are consistent with the model of Fig. 1B. In addition, we measure unidirectional Na\(^+\) influx and efflux under conditions of net forward transport by the carrier. We find a
component of Na\(^+\) influx that behaves as predicted by the model of Fig. 1A, as well as a component of Na\(^+\) efflux that appears to be a Na\(^+\) backleak mediated by the pH\(_i\)-regulating system (Fig. 1B).

Some of these results have been presented to the Biophysical Society (Russell et al., 1982; Russell and Brodwick, 1982).

**MATERIALS AND METHODS**

**Materials**

Giant barnacles from Puget Sound were obtained from David King (Friday Harbor, WA) and kept in an aerated aquarium at 13°C. Only fibers from the *depressor scutorum rostralis* or *lateralis* groups were used. After dissection, the fibers were stored at 6°C in artificial barnacle seawater (BSW) buffered with HEPES (see below). Before cutting them from the shell, the fibers were soaked in 0-Ca BSW (Mg substituted for Ca) to prevent contracture.

SITS was purchased from ICN Nutritional Biochemicals (Cleveland, OH) as the disodium salt. DIDS (4,4' -diisothiocyanostilbene-2,2'-disulfonic acid) was purchased from Pierce Chemical Co. (Rockford, IL) also as the disodium salt. All experiments were conducted at 20°C.

**Solutions**

The normal external bathing fluid is denoted as HEPES-BSW and had the following composition in millimoles/liter: 464 Na\(^+\), 10 K\(^+\), 11 Ca\(^{2+}\), 32 Mg\(^{2+}\), 541 Cl\(^-\), and 30 HEPES (20 mM in the anionic form; pK \(\cong\) 7.5). The pH was 7.8 and the osmolality was 975 mosmol/kg. In some experiments, in which pH\(_o\) was lowered to 6.7 or 6.4, 30 mM piperazine-N,N'-bis-(2 ethanesulfonic acid) (PIPES; pK \(\cong\) 6.8) replaced the HEPES. When choline was used to replace Na\(^+\), it was freshly recrystallized from isopropanol (see Boron et al., 1981). All external solutions contained \(3 \times 10^{-5}\) M ouabain, which was added just before use from a 1 \(\times\) 10\(^{-2}\) M stock solution of ouabain in glass-distilled water.

Two standard internal dialysis fluids (DF) were used, one with a pH of 7.3–7.4, and the other with a pH of either 6.6 or 6.0. The former had the following composition in millimoles/liter: 204.7 K\(^+\), 24.4 Na\(^+\), 7 Mg\(^{2+}\), 30 Cl\(^-\), 174.4 glutamate, 100 HEPES (38.7 in the anionic form), 460 mannitol, 2 EGTA, 0.5 phenol red, and 4.0 ATP; the osmolality was \(\cong\) 1,000 mosmol/kg. The more acidic dialysis fluids had the following composition in millimoles/liter: 207 (pH 6.0) or 227 (pH 6.6) K\(^+\), 24.4 Na\(^+\), 7 Mg\(^{2+}\), 30 Cl\(^-\), 95.4 glutamate, 100 PIPES (13.7 mM in the doubly anionic form at pH 6.0, 38.7 mM at pH 6.6), 460 mannitol, 2.0 EGTA, 0.5 phenol red, and 4.0 ATP; the osmolality was \(\cong\) 1,000 mosmol/kg. In some experiments at low pH, N-(2-acetamido)-2-aminoethanesulfonic acid (ACES; pK \(\cong\) 6.8) was substituted for PIPES. In all cases, the ATP was added to the DF just before the experiment from a 400-mM stock solution titrated to pH 7.0 with KOH and kept frozen until used.

**Internal Dialysis**

Inasmuch as the general methods for the use of internal dialysis to measure both unidirectional influx and efflux have been described in detail elsewhere (e.g., Brinley and Mullins, 1967; Russell and Brodwick, 1979), only significant differences will be mentioned here. The outer diameter of the cellulose acetate tubing
was 253 \mu m and the inner diameter was 171 \mu m. When HCO_3^- was included in the DF, the solution was delivered from a gas-tight syringe (Hamilton Co., Reno, NV) through small-bore (635 \mu m OD; 432 \mu m ID) stainless-steel tubing (Small Parts, Inc., Miami, FL) in an effort to reduce the loss of CO_2 prior to the DF's contact with the barnacle muscle fiber. However, CO_2 loss could not be entirely prevented inasmuch as the DF had to pass through \sim 1.5 cm of CO_2-permeable cellulose acetate dialysis tubing before entering the barnacle muscle fiber. The amount of HCO_3^- loss was estimated by measuring the [HCO_3^-] of the DF exiting the dialysis capillary using a micro-van Slyke apparatus (Harleco, Gibbstown, NJ). We found that at a DF flow rate of 2 \mu l/min, the [HCO_3^-] of the exiting DF was slightly less than 1 mM when the initial [HCO_3^-] was 4 mM. Increasing the flow rate to 10 \mu l/min resulted in a [HCO_3^-] of the exiting fluid of \sim 2.5 mM. When the DF was made to contain 10 mM HCO_3^-, the exiting HCO_3^- concentrations were 1.5 and 6.0 mM, respectively, at flow rates of 2 and 10 \mu l/min. For most of the experiments reported here, the slower flow rate was used. However, for the experiments designed to test the pH sensitivity of Na^+ efflux, the higher flow rate was used. CO_2 loss from external fluids was prevented by their delivery directly to the fiber through CO_2-impermeable glass and stainless-steel tubing.

In all experiments, the pH was measured by means of a Hinke-style glass pH electrode (see below), which was inserted adjacent to the dialysis tube through one end-cannula. An internal reference electrode measuring membrane potential (V_m) was filled with 0.5 M KCl and inserted through the opposite end-cannula. The tips of the two electrodes were positioned as close as possible to one another in the center of the dialyzed region of the fiber. The relationships among the muscle fiber, the dialysis tube, and the two electrodes are similar to those already illustrated for squid giant axons (Boron and Russell, 1983).

**Isotope Techniques**

Sodium-22 was purchased from New England Nuclear (Boston, MA) as a carrier-free solution and added to the experimental solutions. Samples were collected directly into scintillation vials to which a toluene-Triton X-100 cocktail (Nadarajah et al., 1969) was then added. The samples were counted in a Packard model 3330 liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). Several of the external solutions used in these experiments caused significant quenching, requiring appropriate corrections to be made by measuring the quenching of known amounts of the isotope added to the particular BSW.

**pH- and Cl-sensitive Microelectrodes**

The pH-sensitive electrodes were similar to those of Hinke (1967); their fabrication and use have been described elsewhere (Boron and Roos, 1976). The difference in potential between the V_m electrode and the pH electrode was amplified and displayed on a digital voltmeter and, in most cases, a pen recorder. Voltages could be measured with an accuracy >0.5 mV (1 mV \approx 0.01 pH unit). The Cl-sensitive electrodes were of the liquid ion-exchanger type, filled with the Corning 477315 resin (Corning Medical and Scientific, Medfield, MA) (see Russell and Brodwick, 1981).

**Measurements of Intracellular pH**

Previous work (Boron et al., 1978) and results presented below show that in
dialyzed barnacle muscle fibers, unlike dialyzed squid giant axons (Boron and Russell, 1983), the measured pH$_i$ generally is not in equilibrium with the pH of the dialysis fluid (pH$_{DF}$). In particular, when the pH$_{DF}$ is reduced below the normal pH$_i$, the steady state pH$_i$ in the barnacle muscle always exceeds the pH$_{DF}$. The main reason for this is that the acid extrusion rate for barnacle muscle (typical $V_{ax} = 1,000$ pmol$\cdot$cm$^{-2}\cdot$s$^{-1}$) is much greater than that for the squid axon (typical $V_{ax} = 10$ pmol$\cdot$cm$^{-2}\cdot$s$^{-1}$). This observation suggests that the steady state pH$_i$ in the barnacle muscle is determined by the balance between the rate of intracellular acid loading via dialysis (which should be preparation independent) and the rate of acid extrusion by the pH$_i$-regulating system. We estimated the rate of acid loading in an in vitro experiment in which a dialysis tube and a pH electrode were inserted into a glass capillary having an internal diameter of 1,100 $\mu$m, which is comparable to the diameter of an average barnacle muscle fiber. The glass capillary was filled with an "artificial sarcoplasm" (i.e., DF buffered to pH 7.0 with PIPES), and the dialysis tube was then perfused at the rate of 2 $\mu$l/min with the same artificial sarcoplasm buffered to pH 6.0 with PIPES. From the rate of fall in the pH of the artificial sarcoplasm in the glass capillary, taken at a point where this pH was 6.9, we calculated an equivalent net H$^+$ flux from the dialysis tube (i.e., acid-loading rate). When the concentration of PIPES in the two fluids was 100 mM, the acid-loading rate (nominal H$^+$ flux per unit area of dialysis capillary) was 600 pmol$\cdot$cm$^{-2}\cdot$s$^{-1}$, whereas reducing the PIPES concentration to 1 mM reduced the acid-loading rate to 5 pmol$\cdot$cm$^{-2}\cdot$s$^{-1}$. Since the dialysis tube has a diameter of 250 $\mu$m these fluxes across the dialysis tubing are equivalent to trans-sarcolemmal fluxes of 136 and 1.1 pmol$\cdot$cm$^{-2}\cdot$s$^{-1}$, respectively, for an 1,100-$\mu$m-diam muscle fiber. In the dialyzed barnacle muscle (unlike the squid axon), even the faster rate of nominal acid loading can be easily exceeded by the sarcolemmal acid extrusion rate when the pH$_i$ is $\sim$6.9. However, when the acid extrusion process is blocked by SITS, DIDS, or furosemide, the pH$_i$ falls towards pH$_{DF}$. The rate-limiting step in acid-loading muscle fibers by dialysis is probably diffusion across the dialysis tube membrane rather than diffusion through the sarcoplasm (Engasser and Horvath, 1974).

**RESULTS**

**Net Reversal of the pH$_i$-regulating Mechanism**

In the following experiments we continuously dialyzed the fiber, thereby controlling the cellular concentrations of K$^+$, Cl$^-$ (confirmed with liquid ion-selective microelectrodes), and Na$^+$ and supplying ATP, while directly measuring changes in pH$_i$. The pH$_i$ was initially reduced by dialyzing with a pH 6.0 fluid that contained 100 mM PIPES. After reducing the pH$_i$ to the desired level (e.g., pH 6.9), we switched to a DF containing only 10 mM buffer. The reduced buffer concentration resulted in a lower acid-loading rate (see above) and permitted the pH$_i$-regulating system (Fig. 1) to produce relatively large pH$_i$ changes even though the fibers were still being dialyzed. By means of this technique we were able to study the pH$_i$-regulating system running forward and backward under several ionic conditions.
Net Reversal Caused by Reducing pH, and Its Requirement for External Cl-

It is already known that the rate of acid extrusion from acid-loaded barnacle muscle fibers is gradually reduced to nearly zero as the pHo is lowered from 8.6 to 6.8 (Boron et al., 1979). The experiment of Fig. 2 indicates that further lowering the pHo to 6.4 (1 mM HCO₃⁻ 1.6% CO₂) actually reverses the pH-regulating system, resulting in the net uptake of acid (i.e., a fall in pH). At point a, dialysis was begun with a fluid of pH 6.0 (100 mM PIPES), which caused the pH to fall until it began leveling
off at ~6.9. Note that this latter pH is considerably more alkaline than the pH_{DF}, the discrepancy reflecting the balance between acid loading (i.e., dialysis) and acid extrusion (see above). At b, the DF was switched to one buffered to pH 6.9 with only 10 mM PIPES. With the acid-loading rate thus reduced, the pH increased because of the activity of the forward-running pH-regulating mechanism (segment bc). However, when pH_{o} was reduced to 6.4 (1 mM [HCO_{3}^{-}]_{o}/1.6\% CO_{2}), the pH_{i} fell rapidly (cd), presumably because of reversal of the pH-regulating system. Note that at the end of this time (point d) the pH_{i} (6.75) was more acidic than pH_{DF} (6.9). Therefore, the observed intracellular acidification could not have been due simply to a passive relaxation of the pH_{i} to pH_{DF}.

The model in Fig. 1B indicates an external Cl^{-} requirement for reversal of the pH-regulatory transport process. When we replaced external Cl^{-} with gluconate (de), not only was the fall of pH_{i} blocked, but the pH_{i} actually began to increase. SITS blocks the alkalinization induced by Cl^{-}-free treatment (not shown), which provides further evidence that this rise of pH_{i} represents the operation of transporters in the forward mode of the pH-regulatory process. When external Cl^{-} was reintroduced (segment ef), the pH_{i} resumed its fall. At point f, when the pH_{o} was raised to 7.8 by increasing [HCO_{3}^{-}]_{o} to 25 mM (1.6\% CO_{2}), the pH_{i} rose (fg) as the
pH-regulating system returned to the net forward-running mode. When the pH was subsequently returned to 6.4, the pHi again declined (segment gh). Finally, application of 0.5 mM SITS in the BSW blocked both acidification (hi) and the increase of pHi normally evoked by raising the pHo to 7.8 (ij). Thus, the pH-regulating system can apparently mediate both forward and reverse transport, the net direction depending, in this experiment, on the values of pHo and [Cl-].

Net Reversal Caused by Reducing [Na+]o
The well-known dependence on external Na+ of the forward-running pH-regulating mechanism suggests that removing external Na+ should cause reversal of this transport system (see Fig. 1). In the experiment of Fig. 3, a muscle fiber was initially exposed to pH 6.4 BSW (1 mM HCO3/1.6% CO2) in order to hasten acid loading. When dialysis was begun at point a with a fluid of pH 6.0 (100 mM PIPES), the pHi fell relatively rapidly (ab) to a value of ~6.8. Even when the pHDF was raised to 7.0 (10 mM PIPES), the pHi continued to fall (bc) below pH 6.8, as a result of the reversal of the pH-regulating system induced by the acidic pHo. Subsequently increasing the pHo to 7.8 (25 mM HCO3/1.6% CO2) caused pHi to rise rapidly (cd), because of the forward operation of the pH-regulating system. Removal of external Na+ not only halted this intracellular alkalinization (de), but produced a pHi more acidic than pHDF, a result that is consistent with net reversal of the transporter. Although Na+ was replaced by choline in this experiment, similar results have been obtained using either N-methyl-D-glucamine or bis(2-hydroxyethyl)-dimethylamine as Na+ substitutes. The latency between the application of Na-free BSW and the resultant fall of pHi probably reflects the rather slow washout of Na+ from the extracellular space.2 The intracellular acidification produced by Na+ removal was accelerated by the simultaneous reduction of the pHo (ef), which indicates that the effects of altering pHo and [Na+]o are additive. That the acidifying effect of both these changes was mediated by the pH-regulating mechanism is further evidenced by the inhibition of acidification that resulted from treatment with 0.5 mM SITS (fg).

[Na+]o Dependence of Net Reversal
The model of Fig. 1 shows that the reversed mode of the pH-regulating mechanism requires intracellular Na+ and, therefore, that it ought to depend on [Na+]o. Fig. 4 illustrates the effects, on both the reversed and forward-running pH-regulating system, of changing [Na+] on nominally

Given an apparent K for external Na+ of 59 mM ([HCO3]o = 10 mM; pHo = 8.0) for acid extrusion, [Na+]o must be reduced to ~7 mM for the acid extrusion rate to be reduced to 10% of maximal. Since the time constant for the washout of the extracellular space is 7–9 min in barnacle muscle, a delay between removal of Na+ and reversal of the pHi increase is expected.
0 mM to 75 mM. The fiber was dialyzed with a Na-free (Na⁺ replaced with equimolar K⁺) fluid, first with a pH 6.0 DF (100 mM PIPES) (ab) and then with a pH 7.0 DF (10 mM PIPES) (bd). During cd, the pH-regulating system was reversed by lowering pH₀ to 6.4 (1 mM HCO₃⁻/1.6% CO₂). Note the relatively slow fall of pHᵢ. During de, the system was returned to the forward-running (i.e., acid-extruding) mode by raising the pH₀ to 7.8 (25 mM HCO₃⁻/1.6% CO₂). Note the unusually high rate of pHᵢ increase. After a 1.5-h period of dialysis with fluid containing 75 mM Na⁺ (ef and fg), the pHᵢ-regulating system was once again put into the reversed and forward-running modes. The pHᵢ decline (i.e., reversal)

<!-- Diagram -->

**Figure 3.** Reversal of the pHᵢ-regulating mechanism by removal of external Na⁺ (replaced with choline). See text for explanation. Ouabain (3 × 10⁻⁵ M) was present throughout this experiment. The slight increase in pHᵢ near point g probably reflects the beginning of equilibration between sarcoplasmic and dialysis-fluid pH.

evoked by reducing the pH₀ to 6.4 (gh) during dialysis with 75 mM Na⁺ was substantially faster than that obtained earlier during the dialysis with 0 mM Na⁺ (cd), when compared at the same pHᵢ, 6.9. In a total of three similar experiments, the rate of acid uptake during dialysis with 75 mM Na⁺ was 4.1 times faster than during dialysis with 0 mM Na⁺. Conversely, the pHᵢ increase (i.e., acid extrusion) elicited by raising the pH₀ to 7.8 (hi) during dialysis with 75 mM Na⁺ was substantially slower than that observed earlier during dialysis with 0 mM Na⁺ when compared at the same pHᵢ, 6.9. For a total of three fibers, the forward-running acid extrusion rate during dialysis with 75 mM Na⁺ was only 31% of that obtained during dialysis with 0 mM Na⁺. Thus, the forward-running pHᵢ-
regulating system was inhibited by high [Na⁺], whereas the reversed system was stimulated.

Unidirectional Na⁺ Fluxes Associated with the pH₁-regulating Mechanism

The foregoing results confirm that the overall pH₁-regulatory process can be made to run forwards and backwards. The results also indicate an important role for both extra- and intracellular Na⁺ in the two modes of operation of the overall transport process, giving support to the models presented in Fig. 1. These models indicate net transmembrane Na⁺ fluxes associated with each mode of overall transporter operation. However, in the barnacle muscle, there has been no demonstration of sodium fluxes directly associated with the operation of the pH₁-regulatory mechanism in either mode of its operation. The following experiments were designed to characterize unidirectional Na⁺ fluxes mediated by the pH₁-regulatory mechanism and to determine whether they also demonstrate properties of a reversible transport process. Finally, these experiments were designed
to test whether, under conditions of net acid extrusion, a minority of the transporters might be running in the reverse mode.

**Na⁺ Influx**

**Dependence on External HCO₃ and pHᵢ** The model of acid extrusion in Fig. 1A shows a net Na⁺ influx that requires, among other things, the presence of external HCO₃. Such a net influx could be the result of an increased unidirectional Na⁺ influx triggered by a fall of pHᵢ. Table IA summarizes the Na⁺ influx results of 24 experiments in which 6 mM HCO₃⁻ (0.4% CO₂; pH 7.8) was externally applied to fibers whose pHᵢ had first been reduced to an average of 6.83 by dialyzing with a fluid buffered to pH 6.0 with 100 mM PIPES. The introduction of exogenous HCO₃ caused the pHᵢ to increase by a mean value of 0.13, presumably because of stimulation of the forward-running pHᵢ-regulating system. The application of HCO₃⁻ also caused the Na⁺ influx to increase by 58 pmol·cm⁻²·s⁻¹. It is important to recognize that the accompanying increase of pHᵢ secondarily reduces acid extrusion (Boron et al., 1979) and, presumably, the HCO₃⁻-stimulated component of Na⁺ influx. Had we been able to maintain the pHᵢ at ~6.83 throughout the application of HCO₃, the HCO₃⁻-dependent Na⁺ influx should have been even greater. In contrast, when the pHᵢ was normal (i.e., ~7.3), application of 6 mM HCO₃ had little or no effect on either Na⁺ influx or pHᵢ. Fig. 5 illustrates this latter point. When the pHᵢ was initially ~6.85, application of HCO₃⁻ caused the aforementioned increase in both pHᵢ and Na⁺ influx. However, as the pHᵢ was raised by dialysis to ~7.3 in the continued presence of 6 mM HCO₃⁻, the Na⁺ influx declined to the value prevailing before the stimulation of the pHᵢ-regulating system by external HCO₃. Similar results were obtained in four other fibers. This finding is consistent with the results of Boron et al. (1978), which suggested that the acid-extrusion mechanism is activated when the pHᵢ falls below its normal value of ~7.3.

| Table I | Na⁺ Influx* |
|---------|-------------|
| (A) Effect of external HCO₃⁻ | 0 mM HCO₃⁻ | 6 mM HCO₃⁻ | 0 mM HCO₃⁻ | HCO₃⁻-dependent |
| Flux | 130±7 | 193±7 | 137±10 | 58.4±3.0 (n = 24) |
| pHᵢ | 6.83±0.03 | 6.99±0.03 | 6.89±0.07 | — |

| (B) Sensitivity to 0.5 mM SITS | SITS + 6 mM |
|-------------------------------|-------------|
| Flux | 0 mM HCO₃⁻ | 6 mM HCO₃⁻ | HCO₃⁻ | SITS-sensitive |
| — | 124±22 | 172±25 | 104±21 | 67.6±9.4 (n = 12) |
| pHᵢ | 6.93±0.04 | 7.04±0.04 | 6.80±0.09 | — |

* Fluxes are steady-state values, given in pmol·cm⁻²·s⁻¹. Values are means ± SE.
Externally applied 0.5 mM SITS completely abolished the HCO$\text{$_3$}$-stimulated Na$^+$ influx and also caused a slow fall of pH$_i$, as summarized in Table IB. In all cases, the inhibition of the Na$^+$ influx by SITS was at least as large as the previous stimulation of the flux by HCO$\text{$_3$}$. In fact, for most experiments the inhibition by SITS was actually slightly greater than the stimulation by HCO$\text{$_3$}$, presumably because of small amounts of endogenous HCO$\text{$_3$}$ present even when the muscle fibers were bathed in nominally HCO$\text{$_3$}$-free media. There are probably two sources of this endogenous HCO$\text{$_3$}$. First, HEPES-BSW at pH 7.8 contains $\sim$0.5 mM HCO$\text{$_3$}$ when it is equilibrated with room air (0.03% CO$_2$). Second, cellular metabolic processes produce CO$_2$, which diffuses into the extracellular cleft system of the barnacle muscle fiber, further increasing $[\text{HCO}_3]$$_o$. Inasmuch as the acid-extruding mechanism has a nominal $K_m$ for external HCO$\text{$_3$}$ of 4.1 mM (Boron et al., 1981), there is a significant activation of acid extrusion even under nominally HCO$\text{$_3$}$-free conditions.

LACK OF EFFECT OF APPLYING HCO$\text{$_3$}$ INTERNALLY

In the experiment of Fig. 5, we demonstrated that externally applied HCO$\text{$_3$}$ stimulated Na$^+$
intracellular HCO₃ stimulated Na⁺ influx only slightly and also had a rather small effect upon pHᵢ. Both effects may have been the consequence of secondarily increasing the P_{CO₂} in the extracellular clefts and therefore of locally increasing [HCO₃⁻]. In contrast, HCO₃ applied extracellularly resulted in a large stimulation of Na⁺ influx and a substantially more rapid increase of pHᵢ. The effects of both internally and externally applied HCO₃ were reversible and were prevented by pretreatment with SITS or DIDS (not shown).

Note that when 6 mM HCO₃ is applied extracellularly (pHₒ = 7.8/
0.4% CO₂), the calculated [HCO₃⁻] at pH 7.1 is ~1.2 mM, which is about the same as the probable actual [HCO₃⁻] obtained while dialyzing with 4 mM HCO₃⁻ (see Methods). Thus, the stimulation of Na⁺ influx by externally applied HCO₃⁻ is probably due almost exclusively to external HCO₃⁻.

Dependence on Intracellular Cl⁻ Acid extrusion in both squid axons (Russell and Boron, 1976; Boron and Russell, 1983) and snail neurons (Thomas, 1977) is dependent upon the presence of intracellular Cl⁻. If the HCO₃⁻-stimulated Na⁺ influx in the present study is mediated by the acid-extrusion mechanism depicted in Fig. IA, then this Na⁺ influx also ought to require internal Cl⁻. We found that when all the Cl⁻ was removed from the DF (normal [Cl⁻]DF = 30 mM), except for the 10 mM Cl⁻ that contaminated the 100 mM PIPES buffer, there was no inhibition of either acid extrusion (as judged by changes in the pHᵢ) or the HCO₃⁻-dependent Na⁺ influx. To further reduce [Cl⁻], we (a) replaced the PIPES buffer with ACES (pK = 6.88), which is not contaminated with Cl⁻, and (b) removed Cl⁻ from the external solution to prevent the inward leak of Cl⁻. Using Cl⁻-sensitive microelectrodes, we confirmed that a 60-to 80-min period of such treatment is sufficient to reduce the Cl⁻ activity of the sarcoplasm to <2 mM. Fig. 7 illustrates an experiment, typical of three muscle fibers, which tests whether acid extrusion and the HCO₃⁻-dependent Na⁺ influx are blocked when [Cl⁻] is reduced to near zero. The muscle fiber was first predialyzed with a pH 6.6 fluid containing 30 mM Cl⁻; this caused the pHᵢ to fall to new steady state value of ~6.8, while the Na⁺ influx leveled off at ~140 pmol·cm⁻²·s⁻¹. When the Cl⁻ in the BSW was replaced with propionate, there was a sudden fall in pHᵢ because of the influx and subsequent dissociation of propionic acid. In the nominal absence of HCO₃⁻, there was no evidence of increased acid extrusion (i.e., additional Na⁺ influx) in response to this acute intracellular acid load. However, the introduction of 6 mM HCO₃⁻ to the BSW produced both an increase in the pHᵢ and a stimulation of Na⁺ influx. Both observations are consistent with the predictions of Fig. IA. Moreover, removal of internal Cl⁻ caused the pHᵢ to decline toward pHᵢDF and reduced the Na⁺ influx to its pre-HCO₃⁻-stimulated value, which suggests blockage of the pHᵢ-regulating mechanism. Re-addition of Cl⁻ to the DF restored the transporter's net forward activity, as evidenced by the increases in both pHᵢ and Na⁺ influx.

Effects of Reducing pHᵢ. As we have already demonstrated, a reduction of pHᵢ, not only inhibits acid extrusion, it can also produce a net reversal of the pHᵢ-regulating system in barnacle muscle. Such a reversal would be expected to affect those Na⁺ fluxes mediated by the pHᵢ-regulatory mechanism. The models in Fig. 1 predict that in switching from the forward (acid extrusion) to the reverse (acid uptake) modes, the net Na⁺ flux should change from an influx to an efflux. This change of net flux could be accomplished by a reduction of unidirectional Na⁺ influx and/or an increase of unidirectional Na⁺ efflux. We therefore
Intracellular pH and Na Fluxes in Barnacle Muscle

studied the effects on unidirectional Na⁺ influx of varying the pHₐ at a constant [HCO₃]₀ (i.e., at varying P_{CO₂}) in six fibers. Because low-pH BSW causes both a membrane depolarization and an increase of the SITS-insensitive Na⁺ influx, stable Na⁺ influxes were obtained in only two experiments, one of which is illustrated in Fig. 8. This fiber was dialyzed with a fluid of pH 6.2 (earliest portion of dialysis not shown) while exposed to pH 7.8 HEPES-BSW; this caused the pHᵢ to fall to ~7.0 and stabilize. When the pH₀ was reduced from 7.8 to 6.7 by applying BSW that had a [HCO₃] of 3 mM (2.7% CO₂), the pHᵢ fell even further. Some of this pHᵢ decrease was caused by the influx of CO₂ into the cell. However, inasmuch as the CO₂ should have equilibrated within 30 min (Boron, 1977), the pHᵢ decrease occurring after that time was probably

![Diagram of pH changes and Na influx](image-url)
due to reversal of the pH-regulating system. Under these conditions, Na\textsuperscript{+} influx reached a steady value of \( \sim 170 \text{ pmol cm}^{-2} \text{ s}^{-1} \). Subsequently increasing the pH\textsubscript{o} to 7.8 at a constant \([\text{HCO}_3^-]_o \) (0.2\% CO\textsubscript{2}) produced a rapid rise in pH\textsubscript{i}, only a part of which could have been due to the efflux of CO\textsubscript{2}. The rest of the pH\textsubscript{i} increase, as well as the stimulation of Na\textsuperscript{+} influx to \( \sim 230 \text{ pmol cm}^{-2} \text{ s}^{-1} \), was presumably produced by the forward-running pH\textsubscript{i}-regulating system. Both effects were reversed by returning the pH\textsubscript{o} to 6.7. This stimulation of Na\textsuperscript{+} influx produced by raising the pH\textsubscript{o} of 6.7 to 7.8 (i.e., \( \sim 60 \text{ pmol cm}^{-2} \text{ s}^{-1} \)) was presumably much smaller than would have been observed had the pH\textsubscript{i} been prevented from increasing from 6.7 to 7.1, inasmuch as acid extrusion is inversely related to pH\textsubscript{i} (Boron et al., 1979). Thus, the observed increase in Na\textsuperscript{+} influx probably represents an underestimate of the extra Na\textsuperscript{+} influx that would have occurred at a constant, more acidic pH\textsubscript{i}. Although it is not shown here, the stimulation of Na\textsuperscript{+} influx by pH 7.8 BSW was blocked by externally applied 50 µM DIDS. Treatment with DIDS also caused the
pH increase to be limited to the small (0.10–0.15) and rapid (<30 min) passive change expected from a $P_{CO_2}$ decrease. The mechanism by which low pH$_o$ inhibits both acid extrusion and the coupled Na$^+$ influx is unknown. A previous kinetic study (Boron et al., 1981) of acid extrusion in barnacle muscle, however, suggests that the inhibition may not be due to a direct competition between extracellular H$^+$ and Na$^+$.

**EFFECT OF CYCLIC AMP**

Inasmuch as acid extrusion and associated Cl$^-$ fluxes are stimulated by cyclic AMP (cAMP) in barnacle muscle (Boron et al., 1978), a similar effect of cAMP on the HCO$_3^-$-stimulated Na$^+$ influx would be expected. Accordingly, the effect of $10^{-5}$ M cAMP on the HCO$_3^-$-stimulated Na$^+$ influx was tested in four fibers. When cAMP was added to the DF, no effect on Na$^+$ influx was noted unless HCO$_3^-$ was present and the pH$_i$ was acidic. However, in the presence of 6 mM HCO$_3^-$, cAMP caused Na$^+$ influx to increase further by 40–50 pmol·cm$^{-2}$·s$^{-1}$ and caused the pH$_i$ to alkalinize further by $\sim$0.1 pH unit (from 7.07 ± 0.02 to 7.15 ± 0.01). Both of these effects are consistent with a stimulation of the acid-extrusion mechanism. Additional evidence that these two cAMP effects are mediated by the pH$_i$-regulatory mechanism is that both were blocked by SITS.

**Na$^+$ Efflux**

The models of Fig. 1 illustrate the direction of net Na$^+$ fluxes in the two modes of operation of the pH$_i$-regulating mechanism. Such net fluxes represent the algebraic sum of the two unidirectional fluxes. Because the overall process is reversible, it might be expected that under conditions of net acid extrusion (i.e., net Na$^+$ influx) there could exist a small Na$^+$ efflux mediated by pH$_i$-regulatory transporters running "backwards." The following experiments on unidirectional Na$^+$ efflux were conducted under conditions of acid extrusion (i.e., net Na$^+$ influx). The results show that indeed a "reverse" unidirectional Na$^+$ efflux occurs even while the dominant Na$^+$ flux is an influx.

**EFFECT OF ACIDIC pH$_i$ ON OUABAIN-SENSITIVE NA$^+$ EFFLUX**

Inasmuch as a large fraction of Na$^+$ efflux from barnacle muscle is mediated by the ouabain-sensitive Na$^+$ pump (e.g., Nelson and Blaustein, 1980), it is important to determine the sensitivity of the Na$^+$ pump flux to changes of pH$_i$ and to correct for such changes if they occur. Fig. 9 illustrates an experiment in which the pH$_i$ of a muscle fiber (pH$_{DF}$ = 6.15) was lowered from $\sim$7.45 to $\sim$6.85 while superfusing with nominally HCO$_3^-$-free BSW. This acidic pH$_i$ reduced the total Na$^+$ efflux from $\sim$50 to $\sim$33 pmol·cm$^{-2}$·s$^{-1}$. The extracellular application of ouabain ($3 \times 10^{-5}$ M) further reduced the Na$^+$ efflux to $\sim$19 pmol·cm$^{-2}$·s$^{-1}$. Finally, returning the pH$_i$ to $\sim$7.45 in the continued presence of ouabain caused the Na$^+$ efflux to fall still further, to $\sim$9 pmol·cm$^{-2}$·s$^{-1}$. Thus, the ouabain-sensitive Na$^+$ efflux was $\sim$50 − 9 = 41 pmol·cm$^{-2}$·s$^{-1}$ at pH$_i$ 7.45, but only $33 - 19 = 14$ pmol·cm$^{-2}$·s$^{-1}$ at pH$_i$ 6.85. The results of similar experiments on a total of 19 fibers are illustrated in Fig. 10. Both the total (Fig. 10A) and
the ouabain-sensitive (Fig. 10B) Na⁺ efflux fell as the pHᵢ was reduced. The Na⁺ efflux remaining in the presence of ouabain, however, has the opposite pHᵢ dependence. Furthermore, this ouabain-insensitive Na⁺ efflux is substantially reduced by externally applied DIDS (50 μM) (Fig. 10A). Fig. 10C shows that the DIDS-sensitive component of the Na⁺ efflux has the same pHᵢ dependence as the pHᵢ-regulating mechanism, maximal at low pHᵢ and falling toward zero at normal pHᵢ (i.e., ~7.3–7.4). The magnitude of this DIDS-sensitive Na⁺ efflux at low pHᵢ is only ~10% as large as the Na⁺ influx mediated by the pHᵢ-regulating system

![Diagram](image)

**Figure 9.** Sensitivity of Na⁺ efflux to 3 x 10⁻⁵ M ouabain and to changes in pHᵢ. See text for Discussion. Fiber diameter, 1,275 μm.

(e.g., see Fig. 5). Inasmuch as the net operation of the pHᵢ-regulating system is almost certainly in the forward or acid-extruding mode (Fig. 1A) during the course of these experiments, it seems likely that the DIDS-sensitive Na⁺ efflux represents a small Na⁺ backleak through the transporter. These results show the absolute requirement for ouabain treatment while studying Na⁺ fluxes associated with the pHᵢ-regulating mechanism.

**DEPENDENCE OF OUABAIN-INSSENSITIVE NA⁺ EFFLUX ON EXTERNAL HCO₃⁻ AND pHᵢ** The Na⁺ backleak (i.e., DIDS-sensitive Na⁺ efflux), ob-
served above in nominally HCO$_3^-$-free solutions was presumably supported by endogenous levels of HCO$_3^-$. The experiment of Fig. 11 examines the effect of applying external HCO$_3^-$ on this component of Na$^+$ efflux. When the DF, initially at pH 7.3, was switched to pH 6.6, both the pH$_i$ and the Na$^+$ efflux decreased. The subsequent addition of ouabain to the external fluid produced no pH$_i$ change, but further reduced the Na$^+$ efflux (see Fig. 10). When 6 mM HCO$_3^-$(0.4% CO$_2$; pH 7.8) was applied externally, two effects were noted (Fig. 11): (a) the pH$_i$ became more alkaline, which indicated a stimulation of the forward-running pH$_i$-regulating system (i.e., acid extrusion); and (b) the Na$^+$ efflux rose rapidly, reaching a peak in

**Figure 10.** Effect of acidic pH$_i$ on Na$^+$ efflux. (A) Collated data from 19 fibers which were dialyzed to various pH$_i$ values before application of ouabain ($3 \times 10^{-5}$ M). In some cases, Na$^+$ efflux was measured at two different pH$_i$ values in a single fiber before ouabain addition. Filled circles represent data from fibers before ouabain treatment; open circles represent data from fibers after full effect of ouabain; half-filled squares represent data from fibers treated with both ouabain and DIDS. Addition of DIDS ($5 \times 10^{-5}$ M) after ouabain always resulted in a further decline of Na$^+$ efflux, the magnitude of the decline being greater at lower pH$_i$ values. The lines drawn through the data points are least-squares regression lines. (B) The effect of reducing pH$_i$ on the ouabain-sensitive Na$^+$ efflux. This line represents the difference between the regression line labeled "control" and that labeled "ouabain" in A. (C) The effect of reducing pH$_i$ on the DIDS-sensitive, ouabain-insensitive Na$^+$ efflux. This line represents the difference between the regression line labeled "ouabain" and that labeled "ouabain + DIDS" in A.
~9 min, and then relaxing to a steady value that was still substantially higher than that prevailing before addition of HCO₃⁻. The removal of external HCO₃⁻ returned the Na⁺ efflux to its pre-HCO₃⁻ level. Table IIA summarizes these effects for nine fibers. The steady state increase of Na⁺ efflux averaged 9.6 ± 1.2 pmol·cm⁻²·s⁻¹. In three fibers, 0.5 mM SITS was applied during this steady state, causing the net efflux to fall by 10.5 ± 2.3 pmol·cm⁻²·s⁻¹. Thus, SITS blocked all the Na⁺ efflux stimulated by exogenous HCO₃⁻ as well as that presumably supported by endogenous Dialysis fluid

External fluid

**Figure 11.** Effect of 6 mM extracellular HCO₃⁻ on ouabain-insensitive Na⁺ efflux. Reduction of pHᵢ and application of ouabain (3 × 10⁻⁵ M) had their usual effects of reducing Na⁺ efflux. When 6 mM HCO₃⁻ (0.4% CO₂; pH 7.8) was introduced into the external fluid, both Na⁺ efflux and the pHᵢ increased. Na⁺ efflux was greatest when the pHᵢ was most acidic, finally relaxing to a steady value as the pHᵢ rose and stabilized. Both effects were reversed by removal of external HCO₃⁻. Fiber diameter, 1,100 μm.

HCO₃⁻. In three fibers dialyzed to pHᵢ 7.35, addition of 6 mM HCO₃⁻ to the BSW had no effect on the Na⁺ efflux. This last observation, that a component of Na⁺ efflux is inversely related to pHᵢ, suggests an explanation for the transient nature of the stimulation of the Na⁺ efflux by HCO₃⁻. As is evident from Fig. 11, Na⁺ efflux is maximally stimulated at a time when the pHᵢ is relatively low and falls off as the pHᵢ recovers. The same pattern was previously identified for the forward-running pHᵢ-regulating system (Boron et al., 1978, 1979).
EFFECT ON OUABAIN-INSENSITIVE \( \text{Na}^+ \) EFFLUX OF APPLYING \( \text{HCO}_3^- \) INTERNALLY

The preceding experiment demonstrates that a portion of the ouabain-insensitive \( \text{Na}^+ \) efflux is enhanced by the application of external \( \text{HCO}_3^- \). As pointed out above, such a treatment also raises \([\text{HCO}_3^-]_i\). If the \( \text{HCO}_3^- \)-stimulated \( \text{Na}^+ \) efflux represents \( \text{Na}^+ \) movements via pH- regulating transporters running in reverse, the model of Fig. 1B would suggest that it is indeed this internal \( \text{HCO}_3^- \) that stimulates the \( \text{Na}^+ \) efflux. We tested this hypothesis in the experiment of Fig. 12, in which we applied nominally 4 mM \( \text{HCO}_3^- \) internally via dialysis. (As noted in Materials and Methods, such treatment probably resulted in an actual \([\text{HCO}_3^-]_i\) of <1 mM.) Although internally applied \( \text{HCO}_3^- \) might raise the local \([\text{HCO}_3^-]_o\), as well, this effect was probably slight; the \( \text{pH}_i \) was not substantially increased. Nevertheless, the internal application of \( \text{HCO}_3^- \) increased the \( \text{Na}^+ \) efflux by \(~9\) pmol \( \cdot \text{cm}^{-2} \cdot \text{s}^{-1} \). Table IIB summarizes the result of 14 experiments that were similar to that in Fig. 11 except that 0.5 mM SITS was added at the end of each experiment. The mean internal- \( \text{HCO}_3^- \) -stimulated \( \text{Na}^+ \) efflux was 12.1 pmol \( \cdot \text{cm}^{-2} \cdot \text{s}^{-1} \). This flux is very near that observed during the steady state phase of stimulation by extracellular \( \text{HCO}_3^- \) (cf. Table II, A and B). Such agreement is interesting because treatment with external \( \text{HCO}_3^- \) (6 mM; 0.4% \( \text{CO}_2 \); \( \text{pH}_o = 7.8 \)) and treatment with 4 mM \([\text{HCO}_3^-]_o\) probably result in about the same \([\text{HCO}_3^-]_i\), 0.8 and \(~1\) mM, respectively (see above). Thus, unless the carrier is extraordinarily sensitive to the small amount of external \( \text{HCO}_3^- \) present during dialysis with nominally 4 mM \( \text{HCO}_3^- \), it would appear that internal \( \text{HCO}_3^- \) is sufficient to stimulate \( \text{Na}^+ \) efflux.

### Table II

**Na* Efflux**

| Flux (pmol·cm⁻²·s⁻¹) | 0 mM HCO₃⁻ | 6 mM HCO₃⁻ | 0 mM HCO₃⁻ | HCO₃⁻-dependent |
|----------------------|------------|------------|------------|-----------------|
|                      | 17.0±0.9   | 26.6±1.1   | 16.9±0.8   | 9.6±1.2 (n=9)   |
| pH                   | 6.96±0.02  | 7.10±0.02  | 6.99±0.02  |                 |

| Flux (pmol·cm⁻²·s⁻¹) | 0 mM HCO₃⁻ | 4 mM HCO₃⁻ | HCO₃⁻ | SITS-sensitive |
|----------------------|------------|------------|------|---------------|
|                      | 14.0±0.04  | 26.0±1.5   | 13.8±0.5 | 12.6±1.3 (n=14) |
| pH                   | 7.01±0.02  | 7.08±0.02  | 7.02±0.02 |               |

* Fluxes are steady-state values, given in pmol·cm⁻²·s⁻¹. Values are means ± SE.

**DEPENDENCE OF OUABAIN-INSENSITIVE NA* EFFLUX ON [CL⁻]**

If the \( \text{HCO}_3^- \)-dependent \( \text{Na}^+ \) efflux represents a portion of the population of pH- regulating transporters operating in reverse, then it should require not only internal \( \text{HCO}_3^- \) but also external \( \text{Cl}^- \) (see Fig. 1B), just as the
Na⁺ influx requires external HCO₃⁻ and internal Cl⁻. Fig. 13 illustrates an experiment in which the muscle fiber was predialyzed with a DF of pH 6.6. The subsequent addition of 4 mM HCO₃⁻ to the DF caused the Na⁺ efflux to increase from ~16 to ~37 pmol·cm⁻²·s⁻¹. As [Cl⁻]₀ was reduced in a stepwise fashion (replaced with gluconate) in the continuous presence of nominally 4 mM intracellular HCO₃⁻, there was a corresponding decrease in the Na⁺ efflux. In the total absence of external chloride, Na⁺ efflux was actually reduced to a level below that observed before application of exogenous HCO₃⁻, which again suggests that endogenous HCO₃⁻ supports a portion of Na⁺ efflux at low pH. Upon restoring [Cl⁻]₀ to its initial value, Na⁺ efflux promptly recovered. Similar results were obtained in three other fibers. Gluconate has previously been shown to be a satisfactory Cl⁻ replacement in barnacle muscle (Russell and Brodwick, 1981). Although this anion binds calcium, and thereby reduces free [Ca²⁺]₀, we independently showed in experiments on two fibers that the bicarbonate-stimulated Na⁺ efflux is unaffected by removal of external Ca²⁺.

Removal of external Cl⁻ also causes an apparent stimulation of acid

![Figure 12: Effect of altering ouabain-insensitive [HCO₃⁻]₀ on Na⁺ efflux.](image)
extrusion. This is evidenced by the increases of pH that accompany the reductions in [Cl⁻]₀. Whether this stimulation results from inhibition of those transporters operating in the reverse or acid "uptake" mode and/or stimulation of forward transport by removal of chloride ions that may normally compete with external HCO₃⁻ for the transporter in the forward or acid-extruding mode cannot be determined at the present time.

EFFECT OF PH₀ ON OUABAIN-INSENSITIVE NA⁺ EFFLUX  Net reversal of the pH-regulating system, achieved by lowering the pH₀, could be accomplished either by inhibition of the forward mode and/or stimulation of the reverse mode. We have already demonstrated (Fig. 8) that a fall of pH₀ to 6.7 is accompanied by an inhibition of Na⁺ influx, which suggests that reversal involves an inhibition of transporters operating in the forward mode. Whether reversal at low pH₀ simultaneously enhances transport in the reverse direction was examined in three series of experiments. In one approach, which parallels that used to study pH₀ effects...
on Na⁺ influx (e.g., Fig. 8), fibers were exposed to 3 mM external [HCO₃⁻] while the pHₐ was changed from 6.7 to 7.8 and back to 6.7. This requires changing the CO₂ level from 0.2 to 2.7%, which must necessarily result in large changes in cellular [HCO₃⁻]. Although this was not a problem for the influx experiments, since Na⁺ influx is not a direct function of [HCO₃⁻], it clearly is a problem for the Na⁺ efflux experiments. The results of five such experiments, given in Table III, column 1, show that Na⁺ efflux is much larger after treatment with pHₐ 6.7. Two considerations obscure the meaning of these results. First, the two fluxes were obtained at very different pHᵢ values. The higher Na⁺ efflux at pHᵢ 6.7 may have been due to low pHᵢ rather than to low pHₐ. Second, the two fluxes were obtained at very different values of [HCO₃⁻]. The calculated [HCO₃⁻] in pH 7.8 (0.2% CO₂) BSW = 0.7 mM, whereas it is ~3.3 mM in the pH 6.7 (2.7% CO₂) BSW. Thus, both the discrepancy of pHᵢ and that of [HCO₃⁻] would tend to increase Na⁺ efflux independent of any change of pHₐ per se.

Therefore, we studied the effects of two concentrations of intracellularly applied HCO₃⁻ (i.e., 4 and 10 mM). The intracellular application of HCO₃⁻ has at least three advantages. First, the HCO₃⁻ is applied on its presumed side of action. Second, the attendant change of pHᵢ is much less than noted with extracellular HCO₃⁻ application. And third, the discrepancy in [HCO₃⁻] between pHᵢ 7.8 and 6.7 is expected to be less than where HCO₃⁻ is applied extracellularly. The results presented in Table III (columns 2-4) show that in the presence of intracellular HCO₃⁻, reducing the pHₐ has a much larger stimulatory effect on Na⁺ efflux than in the nominal absence of cellular HCO₃⁻. This effect is more pronounced at higher levels of nominal [HCO₃⁻]. Fig. 14 shows one such experiment. Reducing the pHₐ to 6.7 in the nominal absence of HCO₃⁻ increased the Na⁺ efflux only slightly (~7 pmol⋅cm⁻²⋅s⁻¹; see points a and b), while the pHᵢ slowly declined (cf. Table III). When nominally 10

### Table III

**Comparison of HCO₃⁻-Sensitive Na⁺ Effluxes at Normal and Acidic pHₐ**

|             | pHₐ = 7.8 |          |          |          |
|-------------|-----------|----------|----------|----------|
|             | 3 mM [HCO₃⁻] | 0 mM [HCO₃⁻] | 4 mM [HCO₃⁻] | 10 mM [HCO₃⁻] |
| pHᵢ         | 7.15±0.05 | 7.02±0.04 | 7.11±0.04 | 7.22±0.05 |
| Na efflux   | 25.0±1.7  | 18.1±1.0  | 35.0±4.0  | 43.8±4.0  |
| (B) pHₐ = 6.7 |          |          |          |          |
| pHᵢ         | 6.74±0.04 | 6.92±0.05 | 6.90±0.02 | 7.08±0.06 |
| Na efflux   | 132±13.8  | 24.5±2.5  | 47.3±1.5  | 90.0±8.3  |

Numbers in parentheses refer to the number of fibers used in each treatment.
mM [HCO₃]₀ was applied, Na⁺ efflux increased sharply (see points c and d) and the pHᵢ rose slightly. The former effect may reflect stimulation of reversed transport. The rise of pHᵢ is presumably a result of increased [HCO₃]₀ (caused by the movement of CO₂ from cellular to extracellular fluid). When the pHᵢ was increased to 7.8, the pHᵢ rose more rapidly, which reflects an increased rate of acid extrusion (Boron et al., 1979). The increase of pHᵢ also caused a large and sudden fall in Na⁺ efflux, followed by a slower decline. The slower decline in Na⁺ efflux may reflect an inhibition of all transport by the pHᵢ-regulating system caused by the simultaneously occurring rise in pHᵢ. The sudden decrease in Na⁺ efflux, however, occurs before the pHᵢ has had time to increase substantially and probably reflects a direct inhibition of reversed transport by high external pH. The magnitude of the HCO₃⁻-dependent change in Na⁺ efflux brought about by changing the pHᵢ from 6.7 to 7.8 is easily calculated. This pHᵢ transition produced only a 7 pmol·cm⁻²·s⁻¹ change in Na⁺ efflux in the absence of HCO₃⁻ (points a and b, Fig. 14), but a 118 pmol·cm⁻²·s⁻¹ change in the presence of HCO₃⁻ (cf. points c and d). The HCO₃⁻-dependent Na⁺ efflux in this fiber was thus 111 pmol·cm⁻²·s⁻¹. This extra Na⁺ efflux could be blocked by SITS or DIDS (not shown).

**EFFECT OF CYCLIC AMP** Two fibers dialyzed to pHᵢ 6.95 were tested.
for an effect of $10^{-5}$ M cAMP on ouabain-insensitive Na⁺ efflux. The fibers were first treated with nominally 4 mM intracellular HCO₃⁻, which increased Na⁺ efflux from an average of 14.9 to an average of 33.1 pmol cm⁻² s⁻¹. Addition of the cAMP further increased the Na⁺ efflux to an average of 46.8 pmol cm⁻² s⁻¹. This effect was reversed upon removal of cAMP. As had been noted previously (see above and Boron et al., 1978), cAMP treatment also stimulated acid extrusion, causing the pH to rise from an average value of 7.09 to 7.19.

**DISCUSSION**

Reversal of the pH-regulating Mechanism

Our results strongly suggest that the ion transport mechanism responsible for pH₆ regulation in barnacle muscle can operate in both the forward (Fig. 1A) and reverse (Fig. 1B) directions. Previous work on barnacle muscle (see Roos and Boron, 1982), squid axons (see Russell and Boron, 1982; Boron and Russell, 1983), and snail neurons (see Thomas, 1982) had documented in detail some fundamental properties of the forward-running pH₆-regulating system. These include (a) the dependence of acid extrusion on external HCO₃⁻, (b) a net uptake of HCO₃⁻ and/or efflux of H⁺, (c) the dependence on external Na⁺, (d) a net uptake of Na⁺ that amounts to half the net flux of HCO₃⁻/H⁺, (e) the dependence on internal Cl⁻, (f) a net efflux of Cl⁻ that amounts to half the net flux of HCO₃⁻/H⁺, (g) inhibition by stilbene derivatives, and (h) an inverse dependence of the acid extrusion rate on pH₆. From the model of Fig. 1, one would expect the reversed transport system to have comparable properties. As pointed out in the Introduction, earlier studies on barnacle muscle and snail neurons had suggested that the pH₆-regulating system may mediate a net uptake of acid (Fig. 1A) when the pH₆ is sufficiently lowered. The present study confirms and extends these observations. Our approach was to use dialysis to reduce the pH₆ to a moderately low value (i.e., 6.9–7.0) and then to continue dialyzing the muscle fiber with a fluid whose pH closely matched pH₆. Thus, any increase in pH₆ above pH₆F must have been due to acid extrusion (Fig. 1A) rather than to inhibition of an acid-uptake mechanism, whereas any decrease in pH₆ below pH₆F must have been due to the net uptake of acid (e.g., by the mechanism of Fig. 1B) rather than to an inhibition of acid extrusion. We demonstrated that a net uptake of acid is produced by removal of external Na⁺ or by sufficiently lowering pH₆ (i.e., [HCO₃⁻]o) and that the effects of these two maneuvers summate. Furthermore, this net uptake of acid is blocked either by SITS or by removal of external Cl⁻, and is inhibited by the reduction of [Na⁺], and stimulated by the elevation of [Na⁺]. These data strongly suggest that the observed net uptake of acid is mediated by the reversed pH₆-regulating mechanism (Fig. 1B) and not simply by the inward leak of H⁺ or the outward leak of HCO₃⁻.

The results of experiments such as those shown in Figs. 2-4 indicate
that the pH$_i$-regulating mechanism can be put into either the net forward (Fig. 1A) or net reverse (Fig. 1B) direction, depending on the transmembrane gradients for Na$^+$ and HCO$_3^-$/H$^+$. If the transporter derives its energy from the chemical gradients of the transported ions, then the net free-energy change ($\Delta G_{net}$) is the sum of the free-energy changes for the individual ions:

$$\Delta G_{Na} = RT \ln \left( \frac{[Na^+]}{[Na^+]_o} \right) = RT \ln \frac{24.4}{464} = -2.95RT;$$

$$\Delta G_{Cl} = RT \ln \left( \frac{[Cl^-]}{[Cl^-]_o} \right) = RT \ln \frac{541}{30} = +2.89RT;$$

$$\Delta G_{HCO_3} = RT \ln \left( \frac{[HCO_3^-]}{[HCO_3^-]_o} \right) = RT \ln \frac{0.8}{6} = -2.01RT;$$

$$\Delta G_{H} = RT \ln \left( \frac{[H^+]_o}{[H^+]_i} \right) = RT \ln 1.58 \times 10^{-8}/1.26 \times 10^{-7} = -2.08RT;$$

$$\Delta G_{net} = RT \ln \frac{[Na^+]_o [Cl^-]_o [HCO_3^-]_i [H^+]_o}{[Na^+]_o [Cl^-]_i [HCO_3^-]_o [H^+]_i} = -4.15RT.$$

The above example, given for a pH$_o$ of 7.8 and a pH$_i$ of 6.9, predicts that under ionic conditions that are normal, except for an acidic pH$_i$, the pH$_i$-regulating system should run in the net forward direction (i.e., $\Delta G < 0$). When pH$_o$ is reduced to 6.4, as in the experiment of Fig. 2, the calculated $\Delta G_{net}$ comes to $+2.25RT$, correctly predicting the net reversal of the transporter. Similarly, when $[Na^+]_o$ is reduced to 0 mM, as in the experiment of Fig. 3, the calculated $\Delta G$ is $+\infty$, also correctly predicting net reversal. The ease with which the barnacle's pH$_i$-regulating mechanism can be reversed suggests that the extrusion of acid in this preparation is not directly coupled to ATP hydrolysis.

It is of interest to note that, judging from both measurements of acid extrusion with pH microelectrodes (Boron et al., 1979) and measurements of isotopic Cl$^-$ fluxes (Boron et al., 1978), the barnacle's pH$_i$-regulating mechanism turns off at pH$_i$ values more alkaline than $\sim 7.4$, even though there is sufficient energy in the ion gradients to drive the pH$_i$ to $\sim 7.8$ under the conditions noted in the equations above. This indicates that the normal pH$_i$ of $\sim 7.35$ is not simply achieved as a result of a net thermodynamic equilibrium among the four ion gradients. Rather, kinetic factors must be responsible for the inactivation of the pH$_i$-regulating system at higher values of pH$_i$.

**Additional Properties of the pH$_i$-regulating Mechanism Operating in the Net Forward Direction**

The general properties of the pH$_i$-regulating systems of squid, snail, and/or barnacle muscle are listed in the previous section. The present study, however, for the first time demonstrates in barnacle muscle the dependence of the forward-running pH$_i$-regulating system on internal Cl$^-$ (see Fig. 7). The requirements for external HCO$_3^-$/Na$^+$ had been previously identified and quantified (Boron, 1977; Boron et al., 1981). The
present study (see Fig. 4) also shows that the net rate of acid extrusion is increased by lowering [Na+] and decreased by raising [Na+]. The mechanism of these effects is unknown. Perhaps H+ and Na+ compete for internal binding sites on the transporter. Alternatively, changing [Na+] may induce a redistribution of some transporters from the net forward to the net reverse direction or vice versa.

**Na+ Fluxes**

The present experiments, in which Na+ fluxes were obtained simultaneously with pHi measurements, clearly demonstrate that portions of both unidirectional Na+ influx and efflux have several properties in common with the pH-regulating system operating in the net forward and reverse directions, respectively. These properties are summarized in Table IV. The differences between the Na+ influx and efflux coincide with the expected sidedness of the pH-regulating system. For example, internal

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**Table IV**

|                     | Acid extrusion | Ouabain-insensitive Na+ influx | Acid uptake | Ouabain-insensitive Na+ efflux |
|---------------------|----------------|-------------------------------|-------------|-------------------------------|
| HCO₃⁻ dependence    | Yes            | Yes (outside)                 | Yes         | Yes (inside?)                 |
| Cl⁻ dependence      | Yes (inside)   | Yes (inside)                  | Yes (outside) | Yes (outside)                 |
| Na⁺ dependence      | Yes (outside)  | Yes                           | Yes (inside) | Yes                           |
| Acid pH₁             | –              | –                             | +           | +                             |
| Acid pH₁             | +              | +                             | +           | +                             |
| SITS/DIDS            | –              | –                             | –           | –                             |
| Gyclic AMP           | +              | +                             | NT          | +                             |

*+, stimulates; –, inhibits.
NT, not tested.

Cl⁻ is required for the HCO₃⁻-dependent Na⁺ influx, whereas external Cl⁻ is required for the HCO₃⁻-dependent Na⁺ efflux.

**Na⁺ INFLUX** Most of the Na⁺ influx experiments were performed under conditions in which the pH-regulating system is expected to operate in the net forward direction. In the continuous presence of ouabain, the Na⁺ influx was stimulated by addition of external HCO₃⁻, but only when the pH₁ was dialyzed to a value more acidic than normal (normal pH₁, 7.35–7.45). This pH₁ dependence is the same as that previously observed for acid extrusion (Boron et al., 1979). Intracellular HCO₃⁻ had little or no effect on Na⁺ influx. Concurrent with the external HCO₃⁻-stimulated increase in Na⁺ influx was an increase of pH₁, which is indicative of acid extrusion. Both the extra Na⁺ influx and the increase of pH₁ required the intracellular presence of Cl⁻. Further evidence linking
the extra Na\(^+\) influx to the process of acid extrusion is their common stimulation by cyclic AMP, their common inhibition by the disulfonic acid stilbene derivatives SITS or DIDS, and their common inhibition by low pH\(_o\). Taken together, these data strongly suggest that there is a direct relationship between acid extrusion and the HCO\(_3^-\)-stimulated Na\(^+\) influx.

**Na\(^+\) Efflux** Most of the Na\(^+\) efflux experiments were performed under conditions in which the pH\(_r\)-regulating system is expected to operate in the net forward direction. Even though the transporter's net direction was forward, the population of transporters behaved as if some were operating in reverse (Fig. 1B). Thus, in the continuous presence of ouabain, a component of the Na\(^+\) efflux was (a) stimulated by HCO\(_3^-\), (b) blocked by removal of external Cl\(^-\), (c) blocked by SITS or DIDS, and (d) inversely related to pH\(_r\). Note, however, that this component of Na\(^+\) efflux (i.e., \(\sim\)10 pmol \cdot cm\(^{-2}\) \cdot s\(^{-1}\); see Table II) was quantitatively much smaller than the HCO\(_3^-\)-stimulated Na\(^+\) influx (i.e., \(\sim\)58 pmol \cdot cm\(^{-2}\) \cdot s\(^{-1}\); see Table IIA) obtained under identical conditions. Finally, the results presented in Table III and Fig. 14 indicate that a decrease of pH\(_r\) stimulates Na\(^+\) efflux via the pH\(_r\)-regulatory transporters. Thus, net reversal by the overall system apparently can involve inhibition of forward-running transport and enhancement of reverse-mode transport.

**Possible Modes of Operation for the pH\(_r\)-regulating System**

The data of the present study indicate that the barnacle muscle's pH\(_r\)-regulating system can operate in either the net forward or net reversed modes. However, our Na\(^+\) efflux data, as well as the Cl\(^-\) influx data from a previous study (Boron et al., 1978), suggest that even when the transporter is operating in the net forward direction, it can mediate both Na\(^+\) efflux and Cl\(^-\) influx. These results could be accounted for if a small fraction of the population of transporters operate in reverse at any one time. However, the explanation could be even more complicated. As suggested by the model of Fig. 1, the pH\(_r\)-regulating system may have an anionic and a cationic binding site on both the inner and outer faces. If either Na\(^+\) or H\(^+\) can bind to the cationic sites and either Cl\(^-\) or HCO\(_3^-\) can bind to the anionic sites, then 16 modes of transport can be envisaged (see Table V). During normal acid extrusion, mode 1 obviously predominates, but the other 15 could, in principle, contribute to an extent determined by the various substrate concentrations, provided the laws of thermodynamics are not violated. Several modes could mediate Na-Na exchange (5, 8, 15, 16) and several others (6, 7, 9, 12) could mediate Na\(^+\) efflux independent of external Na\(^+\), even when the transporter operates in the net forward direction. In the present study, we have identified an HCO\(_3^-\)-dependent Na\(^+\) efflux, which requires external Cl\(^-\), presumably via mode 6 (i.e., "reversal"). However, we cannot presently rule out the possibility that some of the stilbene-sensitive Na\(^+\) efflux is mediated by other modes of the transporter. A similar analysis could be applied to the Cl\(^-\) influx which also accompanies acid extrusion (Boron et al., 1978).
Previous Studies by Others

Bittar and his colleagues (Bittar et al., 1977, 1979) have studied the effects of external acidification on the Na⁺ efflux from injected barnacle muscle fibers. They found that lowering the pH₆ stimulating Na⁺ efflux, but only when 10 mM HCO₃⁻ was added to the seawater. Furthermore, this response was enhanced by ouabain and by cyclic AMP. They proposed that this Na⁺ efflux is mediated by a hitherto unknown Na⁺-extruding system. However, we suggest that this Na⁺ efflux is mediated by the reverse-mode of the pH-regulating mechanism. Decreasing the pH₆ of a solution to which 10 mM NaHCO₃ is added will increase the PCO₂, both outside and inside the cell, and therefore raise [HCO₃⁻], and lower the pH₆. Indeed, a fall in pH₆ was actually observed (Fig. 4b and Table II in Bittar et al., 1977). Such concurrent changes in [HCO₃⁻] and pH₆ would increase the Na⁺ efflux via the reverse mode of the pH-regulating mechanism. In undialyzed muscle fibers, ouabain ought to further enhance this process by causing an increase of [Na⁺]. Finally, we have shown that cyclic AMP enhances the HCO₃⁻-stimulated Na⁺ efflux.

Effects of pH₆ Changes on the Na-K Pump

The experiments summarized in Fig. 10 demonstrate that the Na⁺ pump, defined as the ouabain-sensitive Na⁺ efflux, is quite sensitive to reductions of pH₆. At the normal pH₆ of 7.3, the ouabain-sensitive Na efflux averaged

| TABLE V |
| Hypothetical Modes of the pH₆-regulating System |

| Intracellular sites | Extracellular sites |
| Exchange process |
| Cation | Anion | Cation | Anion | |
|---|---|---|---|---|
| (A) Acid extrusion modes |
| 1. H⁺ Cl⁻ | Na⁺ HCO₃⁻ | HCl/NaHCO₃ |
| 2. H⁺ Cl⁻ | Na⁺ Cl⁻ | HCl/NaCl |
| 3. H⁺ Cl⁻ | H⁺ HCO₃⁻ | HCl/H₂CO₃ |
| 4. H⁺ HCO₃⁻ | Na⁺ HCO₃⁻ | H₂CO₃/NaHCO₃ |
| 5. Na⁺ Cl⁻ | Na⁺ HCO₃⁻ | NaCl/NaHCO₃ |
| (B) Acid uptake modes |
| 6. Na⁺ HCO₃⁻ | H⁺ Cl⁻ | NaHCO₃/HCl |
| 7. Na⁺ HCO₃⁻ | H⁺ HCO₃⁻ | NaHCO₃/H₂CO₃ |
| 8. Na⁺ HCO₃⁻ | Na⁺ Cl⁻ | NaHCO₃/NaCl |
| 9. Na⁺ Cl⁻ | H⁺ Cl⁻ | NaCl/HCl |
| 10. H⁺ HCO₃⁻ | H⁺ Cl⁻ | H₂CO₃/HCl |
| (C) Isohydric modes |
| 11. H⁺ HCO₃⁻ | Na⁺ Cl⁻ | H₂CO₃/NaCl |
| 12. Na⁺ Cl⁻ | H⁺ HCO₃⁻ | NaCl/H₂CO₃ |
| 13. H⁺ HCO₃⁻ | H⁺ HCO₃⁻ | H₂CO₃/H₂CO₃ |
| 14. H⁺ Cl⁻ | H⁺ Cl⁻ | HCl/HCl |
| 15. Na⁺ Cl⁻ | Na⁺ Cl⁻ | NaCl/NaCl |
| 16. Na⁺ HCO₃⁻ | Na⁺ HCO₃⁻ | NaHCO₃/NaHCO₃ |
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~50 pmol·cm$^{-2}$·s$^{-1}$, whereas it was gradually reduced to zero as the pH$_i$ was reduced to 6.8. This is the first direct demonstration of an acidic pH$_i$ dependence of the Na$^+$ pump in intact cells, and emphasizes the importance of the pH$_i$-regulating mechanism for the control of normal cellular function.

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REFERENCES

Bittar, E. E., B. G. Danielson, W. Lin, and J. Richards. 1977. An investigation of the effects of external acidification on sodium transport, internal pH and membrane potential in barnacle muscle fibers. J. Membr. Biol. 34:223–246.

Bittar, E. E., J. Demaille, E. H. Fischer, and R. Schultz. 1979. Mode of stimulation by injection of cyclic AMP and external acidification of sodium efflux in barnacle muscle fibers. J. Physiol. (Lond.). 296:277–289.

Boron, W. F. 1977. Intracellular pH transients in giant barnacle muscle fibers. Am. J. Physiol. 233:C61–C73.

Boron, W. F., and A. Roos. 1976. Comparison of microelectrode, DMO and methylamine methods for measuring intracellular pH. Am. J. Physiol. 231(3):799–809.

Boron, W. F., and J. M. Russell. 1983. Stoichiometry and ion dependence of the intracellular-pH-regulating mechanism in squid giant axons. J. Gen. Physiol. 81:373–399.

Boron, W. F., W. C. McCormick, and A. Roos. 1979. pH regulation in barnacle muscle fibers: dependence on intracellular and extracellular pH. Am. J. Physiol. 237:C185–C193.

Boron, W. F., W. C. McCormick, and A. Roos. 1981. pH regulation in barnacle muscle fibers: dependence on extracellular sodium and bicarbonate. Am. J. Physiol. 240:C80–C89.

Boron, W. F., J. M. Russell, M. S. Brodwick, D. W. Keifer, and A. Roos. 1978. Influence of cyclic AMP on intracellular pH regulation and chloride fluxes in barnacle muscle fibres. Nature (Lond.). 276:511–513.

Engasser, J.-M., and C. Horvath. 1974. Buffer facilitated proton transport. pH profile of bound enzymes. Biochim. Biophys. Acta. 358:178–192.

Hinke, J. A. M. 1967. Cation-selective microelectrodes for intracellular use. In Glass Electrodes for Hydrogen and Other Cations. G. Eisenman, editor. Marcel Dekker, New York. 464–477.

Keifer, D. W. 1979. Ion requirements for bicarbonate transport in barnacle muscle fibers. Biophys. J. 25:99a. (Abstr.)

Nadarajah, A., B. Leese, and G. F. Joplin. 1969. Triton X-100 scintillant for counting calcium-45 in biological fluids. Int. J. Appl. Radiol. Isot. 20:733–739.

Nelson, M. J., and M. P. Blaustein. 1980. Properties of sodium pumps in internally perfused barnacle muscle fibers. J. Gen. Physiol. 75:183–206.
Roos, A., and W. F. Boron. 1982. Regulation of intracellular pH in barnacle muscle. In Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions. R. Nuccitelli and D. W. Deamer, editors. Kroc Foundation Series. Alan R. Liss, Inc., New York, 15:205–219.

Russell, J. M., and W. F. Boron. 1976. Role of chloride transport in regulation of intracellular pH. Nature (Lond.). 264:73–74.

Russell, J. M., and W. F. Boron. 1982. Intracellular pH regulation in squid giant axons. In Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions. R. Nuccitelli and D. W. Deamer, editors. Kroc Foundation Series. Alan R. Liss, Inc., New York. 15:221–237.

Russell, J. M., and M. S. Brodwick. 1979. Properties of chloride transport in barnacle muscle fibers. J. Gen. Physiol. 73:343–368.

Russell, J. M., and M. S. Brodwick. 1981. Cyclic AMP-stimulated chloride fluxes in dialyzed barnacle muscle fibers. J. Gen. Physiol. 78:499–520.

Russell, J. M., and M. S. Brodwick. 1982. Na fluxes associated with internal pH regulation in barnacle muscle. Biophys. J. 37:235a. (Abstr.)

Russell, J. M., W. F. Boron, and M. S. Brodwick. 1982. Evidence for reversal of acid extrusion in barnacle giant muscle fibers. Biophys. J. 37:234a. (Abstr.)

Thomas, R. C. 1977. The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. J. Physiol. (Lond.). 273:317–338.

Thomas, R. C. 1980. Reversal of the pH regulating system in a snail neurone. Curr. Top. Membr. Transp. 13:23–29.

Thomas, R. C. 1982. Snail neurones intracellular pH regulation. In Intracellular pH: Its Measurement, Regulation and Utilization in Cellular Functions. R. Nuccitelli and D. W. Deamer, editors. Kroc Foundation Series. Alan R. Liss, Inc., New York. 15:189–204.