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pH Regulation in Tissue-Cultured Bovine Lens Epithelial Cells

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Summary. The intracellular pH (pH_i) of tissue-cultured bovine lens epithelial cells was measured in small groups of 6 to 10 cells using the trapped fluorescent dye 2',7'-bis-(2-,carboxyethyl)-5 (and 6)carboxyfluorescein (BCECF). When perifused at 35°C with artificial aqueous humour solution (AAH) containing 16 mM HCO_3^- and 5% CO_2, pH 7.25, pH_i was 7.19 ± 0.02 (SEM, n = 95). On removing HCO_3^- and CO_2 there was an initial transient alkalinization followed by a fall in pH to a steady value of 6.97 ± 0.03 (SEM, n = 54). Addition of 0.25 mM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS) to AAH containing HCO_3^- and CO_2 led to a rapid and pronounced fall in pH. Exposure to Na^+-free AAH again led to a marked fall in pH_i, but in this case the addition of DIDS did not produce a further fall. Substitution of the impermeant anion gluconate for Cl^- in the presence of HCO_3^- led to a rise in pH_i, while substitution in the absence of HCO_3^- led to a fall in pH_i. The above data indicate a significant role for a sodium-dependent Cl^--HCO_3^- exchange mechanism in the regulation of pH_i. Addition of 1 mM amiloride to control AAH in both the presence and absence of HCO_3^- led to a marked fall in pH_i, indicating that a Na^+/H^+ exchange mechanism also has a significant role in the regulation of pH_i. There is evidence for a lactic acid transport mechanism in bovine lens cells, as addition of lactate to the external medium produced a rapid fall in pH_i. Larger changes in pH_i were observed in control compared to HCO_3^--free AAH and in the latter case a pronounced alkalinizing overshoot was obtained on removing external lactate. Tissue-cultured bovine lens cells thus possess at least three membrane transport mechanisms that are involved in pH regulation. The buffering capacity of the lens cells was measured by perturbing pH_i with either NH_4^+ or procaine. The values obtained were similar in both cases and the intrinsic buffering capacity measured in the absence of external HCO_3^- was 5 mM/pH unit (procaine). However, in the presence of HCO_3^- and CO_2 the buffer capacity increases approximately fourfold, indicating that HCO_3^- is the principal intracellular buffer.

Key Words Lens pH · pH regulation · bicarbonate · BCECF · Cl^--HCO_3^- · exchanger

Introduction

The lens must continually and actively extrude protons for two main reasons. Firstly, in common with most cells, the lens has a relatively high negative resting potential and thus there is an inwardly-directed electrical gradient for mobile positive charge. Secondly, as metabolism is largely anaerobic, there is a continuous production of H^+ from lactic acid.

Earlier studies on the rat lens using ion-sensitive microelectrodes showed that the pH in the outermost cells was in the region of 7.0 (Bassnett & Duncan, 1986) and hence the lens must actively extrude H^+. Dynamic studies of pH regulation in the whole lens are limited by the syncitial nature of the lens and long diffusion paths from the external surface. However, epithelial monolayer (Bassnett, 1990) and tissue culture techniques (Stewart et al., 1988) have been developed to overcome this problem.

Most of the previous studies have been carried out either on the amplibian (Wolosin, Alvarez & Candia, 1988) or the chick lens epithelial system (Bassnett, 1990) using the powerful and convenient fluorimetric dye assay technique. For example, Wolosin et al. (1988) showed that Na^+-H^+ exchange was the most important regulatory mechanism in the amphibian lens and, furthermore, that this was activated by a change in lens volume. Bassnett (1990) confirmed both of these findings in the isolated chick lens epithelium but pointed out that Cl^--HCO_3^- exchange also had a role to play. In the toad lens epithelial cells, however, Wolosin, Alvarez & Candia (1989) could find no evidence of Cl^--HCO_3^- exchange, although they suggested that a Na^+-HCO_3^- cotransporter was present in their system.

In the present study fluorimetric assay techniques were applied to mammalian lens cells. In particular the role of sodium-dependent Cl^--HCO_3^- exchange in pH regulation was investigated since a SITS inhibitable, Na^+-dependent increase in $^{36}Cl^-$ efflux has been shown to occur in the mammalian lens in response to an acid stress (Duncan et al. 1992). A possible role for a lactate-transporting mechanism in lens cell pH regulation was also inves-

tigated as Bonanno (1990) has reported the presence of such a system in rabbit corneal epithelial cells.

Materials and Methods

Tissue Culture

Whole bovine eyes were obtained from a local abattoir and the lens dissected out within 4–6 hr of death of the animal. The epithelium was then placed in a 35-mm plastic culture dish and covered with 2 ml of Eagle's Minimal Essential Medium (EMEM) (10% fetal calf serum (FCS)) to initiate the primary explant culture. Confluent cultures were obtained within 2–3 weeks. The cells were then trypsinized and subcultured by placing a drop of medium containing 2×10^4 cells on the center of a clean plastic dish and leaving it to settle for 24 hr. A further 2 ml of EMEM were added and the cells allowed to proliferate for 3 days. At the end of this period the cells were confluent and ready for use.

Experimental Solutions

In order to make changes in the concentrations of external Na^+, Cl^- and HCO_3^-, the cells were equilibrated in a relatively simple artificial aqueous humour (AAH) solution for at least 30 min before the start of any experimental protocol. Control AAH had the following composition (in mM): 124 NaCl, 5 KCl, 0.5 $MgCl_2$, 1 $CaCl_2$, 10 HEPES (N-(2-hydroxyethyl) piperazine-N^1-(2-ethanesulphonic acid)), 5 glucose and 16 $NaHCO_3$. This solution was gassed with 5% CO_2/95% O_2 and adjusted to pH 7.25.

Sodium-free solutions contained 124 mM N-methyl-D-glucamine and 16 mM choline bicarbonate, and the pH was set to 7.25 by adding HCl. Chloride-free solutions contained equivalent amounts of D-gluconate in place of chloride.

Bicarbonate-free solutions were ungassed and 16 mM $NaHCO_3$ was replaced by 16 mM NaCl in the control solution, by 16 mM N-methyl-D-glucamine chloride in the Na^+-free experiments and by 16 mM sodium gluconate in the Cl^--free experiments.

In experiments where the effects of L(+) lactate, procaine and ammonium chloride were studied, equimolar amounts of NaCl were omitted to accommodate the test substance in each case. All solutions were equilibrated, and pH was adjusted at 35°C.

Dye Loading and Fluorescence Measurements

On removal from the CO_2 incubator (35°C), the tissue-cultured cells were washed twice with 4 ml control AAH and then incubated for 30 min in 4 ml control AAH to which 5 μM of the fluorescent dye 2′,7′-bis (carboxyethyl)-5,6-carboxyfluorescein acetoxymethylester (BCECFam) had been added. The cells were finally incubated in fresh AAH for at least 30 min in the presence or absence of HCO_3^- (depending on the experimental protocol) to permit complete deesterification of the dye to the membrane impermeant BCECF form.

A plastic insert was then sealed into the culture dish with inlet and outlet ports to allow rapid perifusion of the central region of cells (approximately 1 cm^2). The time for solution turnover in the central chamber was approximately 1 sec. The experimental solutions were prewarmed before entering the chamber mounted on the heated stage of an inverted microscope (Zeiss IM35), and the cells were maintained at a constant temperature of 35°C. The central cells were irradiated alternatively with 495 and 440 nm light and fluorescence from the trapped dye was measured at 510 nm. The necessary monochromators, dichroic filters and photometers to produce and detect the fluorescence from approximately 6 to 10 cells in the field of view were part of a Photon Technology International (PTI) Deltascan system, and the software systems to control the monochromators and both acquire and process the data were also supplied by PTI.

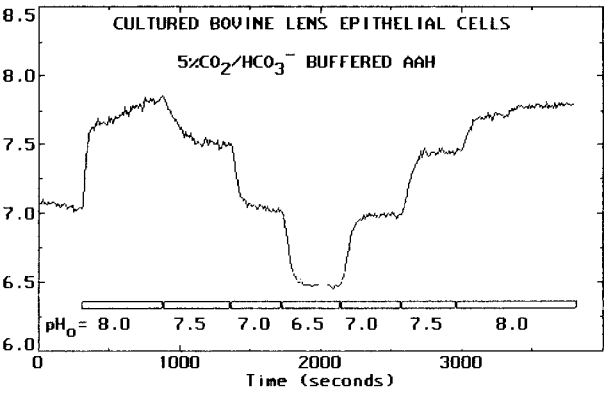

Fig. 1. Calibration of pH_i in bovine lens cells. *See* Materials and Methods section for further details.

At least four replicate experiments of each protocol were carried out on separate dishes of cells. At the end of each experiment the cells were perifused with 10 μM nigericin dissolved in a high potassium (150 mM) buffer. Nigericin is a K^+-H^+ exchanger and equilibrates the internal pH with the known external pH. The pH standard contained 10 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) at pH 6.5, otherwise all standards contained 10 mM HEPES. As the response ratio was linear in the range 7.5 to 6.5, a simple transformation was performed to obtain the corresponding pH_i values from the ratios (Fig. 1). pH_i values outside this range should be taken as approximations, but in fact most of the experimental protocols gave values within the linear range. Where appropriate the results are expressed as mean ± SEM (number of dishes).

Results

The resting pH of tissue-cultured bovine lens epithelial cells was found to be very sensitive to alterations in external CO_2 and HCO_3^-. For example, when CO_2 and HCO_3^- are removed from the medium, there is a rapid alkalinization of pH_i, presumably corresponding to a rapid loss of CO_2, and this is followed by a slower acidifying phase probably due to the loss of internal HCO_3^- (Fig. 2). On return to control medium, the reverse occurs. Due to these transient changes in internal pH, in order to investigate the effect of CO_2 and HCO_3^- on the resting pH_i it was necessary to pre-equilibrate the cells in the appro-

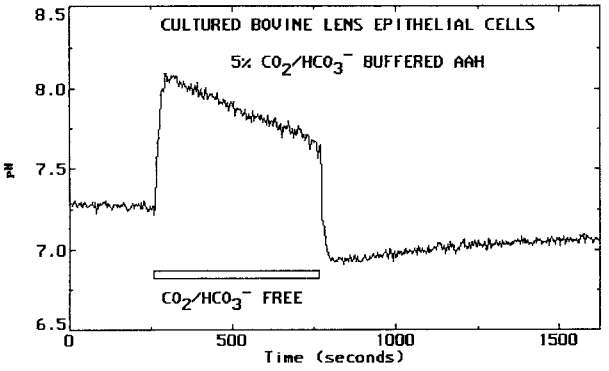

Fig. 2. Transient pH_i response obtained on removing CO_2 and HCO_3^- from the perifusing medium. The initial alkalinization is due to the very rapid diffusion of CO_2 from the cells. Note that all figures give a representative response of experiments carried out on at least four separate dishes of cells.

priate medium. Cells equilibrated for approximately 1 hr in a 5% CO_2/16 mM HCO_3^- medium maintain their pH_i at 7.19 ± 0.02 (n = 95) compared with the value of 6.97 ± 0.03 (n = 54) for cells equilibrated in a HCO_3^- and CO_2-free medium. These results indicate that HCO_3^- has a significant role to play in maintaining a relatively high internal pH.

Stilbene derivatives, such as 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid (SITS) and DIDS, block a range of HCO_3^--transporting mechanisms (Thomas, 1984) and perifusion of bovine lens cells with 0.25 mM DIDS led to a rapid and sustained fall in pH_i (Fig. 3*A*). Although this indicates that an inwardly-directed HCO_3^- movement had indeed been blocked, it was surprising to find that DIDS also produced an internal acidification when CO_2/HCO_3^- had been removed from the external medium (Fig. 3*B*).

HCO_3^- influx is, in many systems, coupled to the efflux of Cl^-, and such a coupling through an anion transporting system could be seen in two types of experiments where the Cl^- gradient across the cell membrane was altered. When external Cl^- was replaced by the impermeant gluconate ion, a marked and reversible alkalinization was observed in the presence of external HCO_3^- (Fig. 4*A*). However, in HCO_3^--free medium a reversible acidification occurred on replacing external Cl^- (Fig. 4*B*).

Evidence for a further pH regulating system in bovine lens cells was obtained by perifusion containing amiloride (1 mM) which blocks Na^+/H^+ exchange. In this case not only is the acidification marked but it is also totally reversible (Fig. 5), in contrast to the partial recovery on removing DIDS (Fig. 3). Furthermore, an acidification was obtained with amiloride also in the presence of HCO_3^- (*data not shown*).

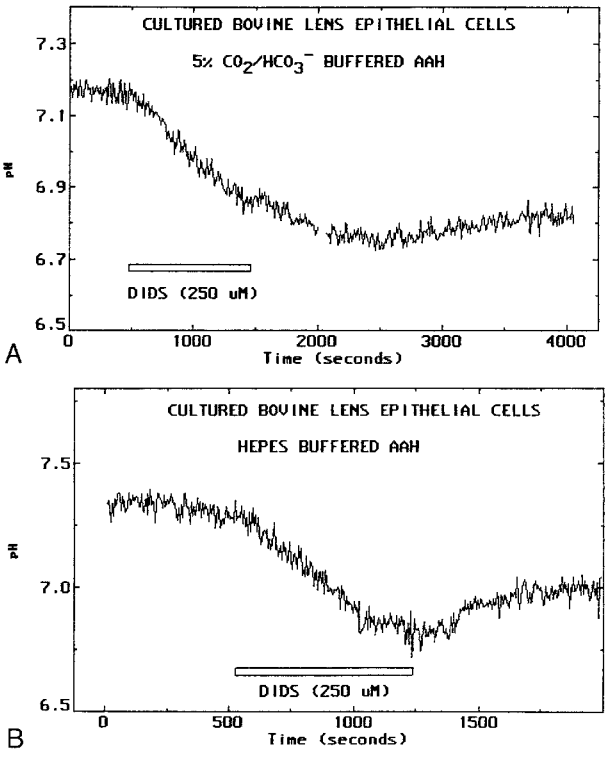

Fig. 3. Effect of adding DIDS (250 μM) on pH_i of bovine cells perifused with (*A*) control and (*B*) HCO_3^- free AAH. Note that in both cases an acidification is obtained, but that only partial recovery is obtained on removing DIDS.

The importance of external Na^+ in regulating pH_i can be seen in experiments where Na^+ is substituted by N-methyl-D-glucamine$^+$. Figure 6 illustrates the effect of perifusing with a Na^+-free medium, while maintaining the normal level of HCO_3^-. A relatively rapid and reversible acidification is observed. A similar time course in the change of pH_i is observed in the absence of HCO_3^- and, interestingly, although the initial starting value for pH_i is different in the two solutions, exposure to sodium-free conditions leads to similar final values of 6.61 ± 0.06 (n = 4) and 6.69 ± 0.01 (n = 4) in the presence and absence of HCO_3^-, respectively.

Exposure to Na^+-free conditions also changes the shape of the pH transients observed when switching from AAH containing HCO_3^- to AAH buffered with HEPES alone. After removing CO_2 and HCO_3^- from the external medium, the pH normally rapidly declines after the initial alkalization (Fig. 2). However, when the switch is made in Na^+-free media the pH response is more rectangular in shape (Fig. 7).

Further evidence for a crucial role for Na^+ can also be seen in experiments where an internal acidification has been induced. The most convenient way

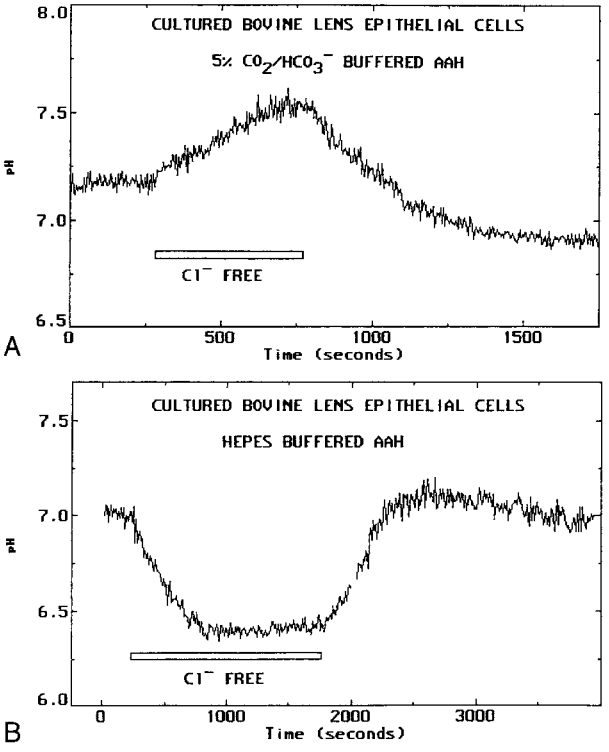

Fig. 4. Effect on pH_i of removing external chloride in the presence (*A*) and absence (*B*) of external HCO_3^-.

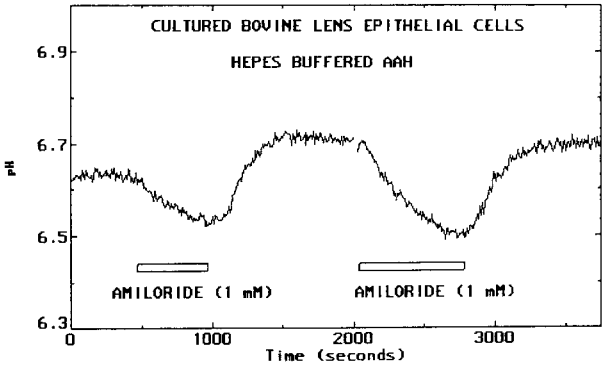

Fig. 5. Effect of amiloride (1 mM) on cells perifused with HEPES AAH. Note that, unlike exposure to DIDS (Fig. 3), the inhibition is quite reversible.

of perturbing pH_i without changing the external pH is by the well-characterized NH_4Cl prepulse technique (Boron & De Weer, 1976). During exposure to NH_4Cl, there is a rapid alkalinization and on removal, there is an acid shift. In control AAH, there is a rapid recovery after the acid shift, while removal of external Na^+ almost totally inhibits recovery (Fig. 8). Replacing Na^+ restores the ability to recover.

NH_4Cl prepulse experiments can also be used to determine the buffering capacity of the cells. On

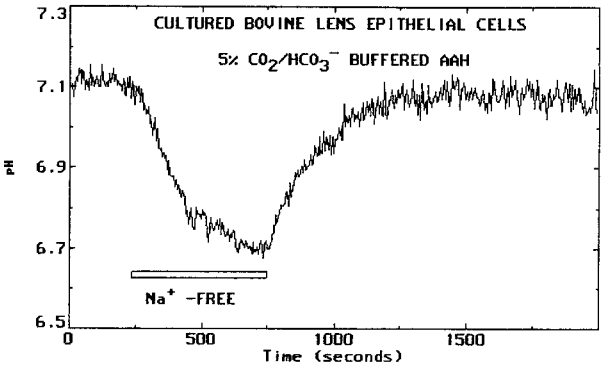

Fig. 6. Effect of removing external sodium on pH_i.

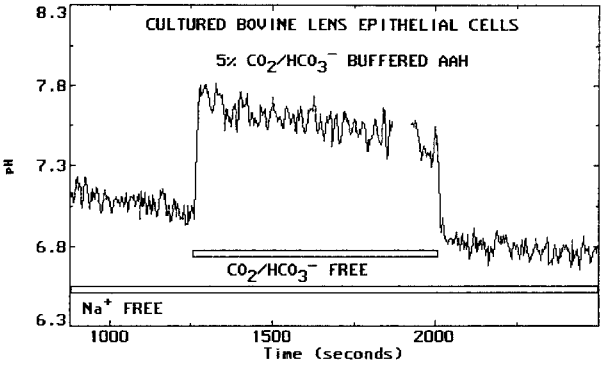

Fig. 7. Response of pH_i to removal of CO_2/HCO_3^- while perifusing with Na^+-free medium. The steady decline in the baseline is due to the acidifying effect of sodium removal. Note, however, that despite this the transient nature of the CO_2 response is much less pronounced than in control medium (*cf.* Fig. 2).

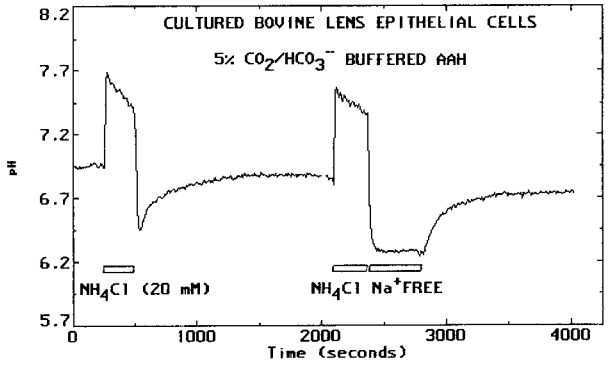

Fig. 8. Effect of pH_i on perifusing with AAH containing 20 mM NH_4Cl. Note that on return to control solution there is a pronounced acidification with eventual recovery to near the control value. This recovery phase is totally abolished on removing external sodium (second response).

removal of NH_4Cl, coincident Na^+-free conditions ensure that there are no pH-regulating mechanisms in operation opposing the acid load. The measured acid shift can then be used to calculate the buffering capacity using the equation (Szatkowski & Thomas, 1989).

$$\beta = \frac{C \cdot 10^{(pK - pH_i)}}{[1 + 10^{(pK - pH_o)}] \cdot \delta pH_i} \quad (1)$$

where β is the buffering capacity ($mM \cdot pH^{-1}$), C is the extracellular concentration of NH_4Cl (10 mM), pH_i is the value of internal pH taken immediately before the removal of NH_4Cl, pH_o is the pH of the bathing medium, pK is that for the acid dissociation of NH_4^+, (8.95) and δpH_i is the change in pH on removing the NH_4Cl. The buffering capacities determined in control and HCO_3^- free solutions were 30.2 ± 4.4 ($n = 4$) and 8.2 ± 0.5 ($n = 4$) $mM \cdot pH^{-1}$, respectively. Another method was also employed. Szatkowski and Thomas (1989) have previously shown that the weak base procaine gave a reliable measure of buffering capacity in snail neurones, and this method was applied in the present system (Fig. 9). The symmetric form of a procaine (5 mM) pulse suggests that bovine tissue-cultured cells are much less permeable to the protonated form of procaine than NH_4^+ (*compare* Figs. 8 and 9). This permits the changes in pH_i observed both on adding and removing procaine to be used to calculate the buffering capacity. As the determination of β implies the absence of any pH regulatory mechanisms apart from intracellular buffer systems, the experiments were carried out in the absence of external Na^+ and the presence of DIDS (0.25 mM). Since these conditions produced an internal acidification, while procaine itself alkalinized the cell, an estimate of the buffering capacity could, in this case, be obtained closer to physiological conditions. The values obtained for β were 27.1 ± 3.6 ($n = 4$) and 5.34 ± 0.19 ($n = 4$) in the presence and absence of HCO_3^-, respectively. It should be noted that procaine solutions are slightly yellow in color, and produce a reduction in signal with BCECF both at 495 and 440 nm. Fortunately, the reductions are in proportion so that procaine does not, in fact, perturb the ratio.

Addition of lactate at constant external pH produces an internal acidification presumably by entering via a carrier mechanism. The addition of lactate (10–40 mM) to control medium rapidly reduces pH_i in a dose-dependent manner (Fig. 10*A*), while addition of the same concentration in the absence of HCO_3^- produces smaller responses throughout the range (Fig. 10*B*). The small shifts are surprising in view of the decreased buffering capacity in the absence of HCO_3^-. Furthermore, the responses are distinctly biphasic in nature in the absence of HCO_3^- (Fig. 10*B*). However, addition of 1 mM amiloride to the HCO_3^- free medium greatly reduces the biphasic nature of the response (Fig. 11). In some systems, lactate transport is also coupled to Na^+, but this does not seem to be the case in lens cells, as lactate still induced an acid shift when external Na^+ was replaced by N-methyl-D-glucamine (*data not shown*).

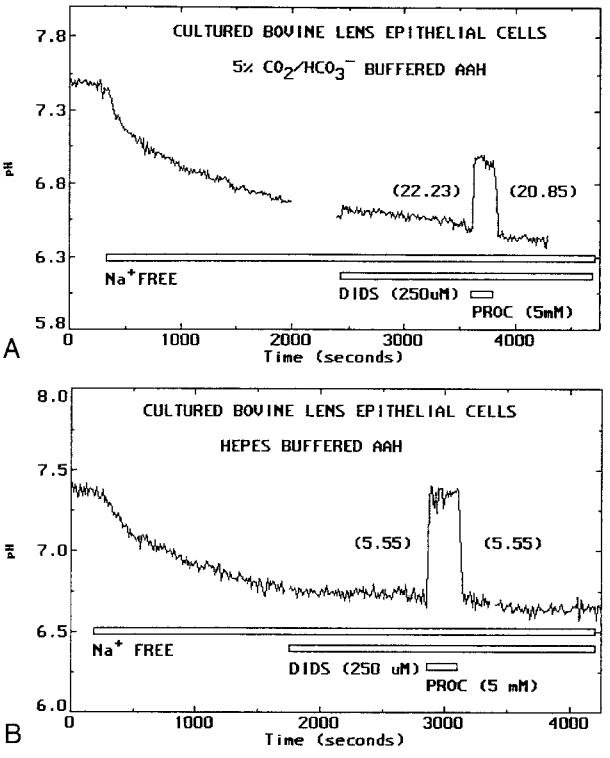

Fig. 9. Internal buffer capacity obtained by the procaine method. Note that Na^+-free conditions are maintained throughout the measurement. DIDS (250 μM) has also been added to ensure complete absence of exchange mechanisms. In this case it is possible to obtain estimates for β both at the beginning and end of the procaine pulse and the values (in parentheses) are much higher in the presence (*A*) compared with the absence (*B*) of HCO_3^-.

Discussion

The internal pH measured in bovine tissue-cultured cells in the presence of HCO_3^- is close to the values previously reported for the whole rat lens (Bassnett & Duncan, 1985) and human tissue-cultured lens cells (Stewart et al., 1988). Internal values are slightly acid with respect to the bathing medium, in contrast to the values obtained from chick (Bassnett, 1990) and amphibian epithelial cells (Wolosin et al.,

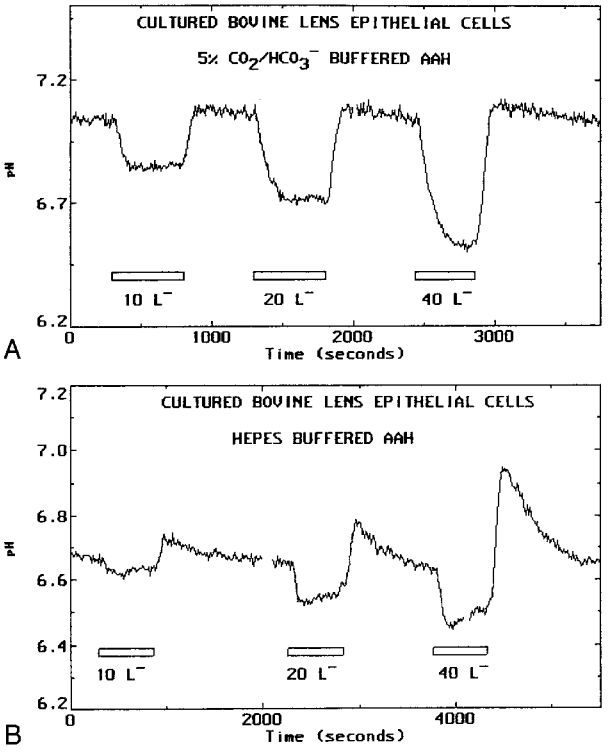

Fig. 10. Characteristics of the response to external lactate. Lactate concentration is given in mM. Note that in HEPES-buffered AAH (*B*), the responses are clearly biphasic in nature throughout the range, while in control AAH (*A*) the responses are more rectangular, although a slight overshoot is present at the "off" phase at the higher concentrations.

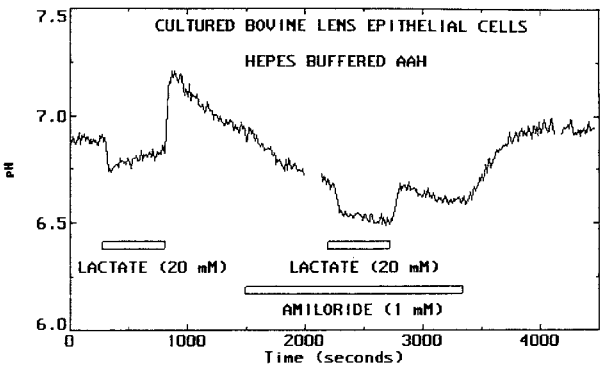

Fig. 11. Effect of amiloride on the lactate response. The first response clearly shows the biphasic characteristics of the response obtained when lactate is applied in a bicarbonate-free medium. Addition of amiloride initiates a rapid acidification, and exposure to lactate does not produce a pronounced biphasic response.

1988), where values were found to be slightly alkaline with respect to the external medium. In both chick (Bassnett, 1990) and bovine cells removal of HCO_3^- is accompanied by a pronounced acidification on equilibration. Interestingly, in the amphibian system removal of HCO_3^- led to a maintained alkalinization and Wolosin et al. (1988) suggested that the HCO_3^- system regulating pH was not operational at high pH values. In chick and bovine cells, however, it appears that a HCO_3^- system does contribute to the maintenance of a high pH in the resting state.

The alkalinizing effect of external HCO_3^- seen in both chick and bovine cells is most likely to occur via a carrier-mediated inward movement of HCO_3^-. The two likeliest candidates are the simple Na^+-HCO_3^- cotransporter and the more complex quadruple ion exchanger where the inward movement of Na^+ is coupled to the movement of HCO_3, Cl^- and H^+. The simple Na^+-HCO_3^- cotransporter does, indeed, appear to exist in eye tissues, but its presence has been implicated in fluid transport rather than in pH regulation (Di Mattio, Degnan & Zadunaisky, 1983; Helbig et al., 1988). The major evidence for the participation of a coupled Na^+-H^+-Cl^--HCO_3^- system in pH regulation of the lens comes from the recent work of Duncan et al. (1992) on the rat lens. In that system, $^{36}Cl^-$ efflux from the lens was stimulated following a range of acid loads, and the increase could be abolished either by the removal of external Na^+ or the addition of SITS. No pH measurements were carried out on the rat lens, but the present study shows that application of DIDS (250 μM) to control AAH does indeed produce a pronounced acidification (Fig. 3*A*). The rat lens data also suggested that the quadruple exchange mechanism could operate in either HCO_3^-/Cl^- or Cl^-/Cl^- exchange mode and there is evidence that this could occur in the bovine cells. Figure 3*B*, for example, shows that DIDS still produces an acidification in the absence of HCO_3^-. In this case DIDS would be inhibiting the exchanger acting in the Na^+-H^+-Cl^--Cl^-mode. It is important to note that, even when operating in a HCO_3^--free medium, the exchanger is still capable of acid regulation through the expulsion of H^+. Such an ability would also explain the difference in pH change observed on removing external Cl^- in the presence and absence of HCO_3^- (Fig. 4*A* and *B*). In the former case (Fig. 4*A*) removal of external Cl^- removes competition in the exchanger between external Cl^- and HCO_3^-, permitting a more rapid entry of HCO_3^- and a consequent alkalinization. When the same substitution is made in the absence of HCO_3^-, then the exchanger operating in the Cl^--Cl^- exchange mode is blocked and hence removal of H^+ is inhibited and a pronounced acidification ensues (Fig. 4*B*). These two modes of operation have been previously well documented in invertebrate neurones (Boron, Russell & Brodwick, 1978). If, however, the quadruple exchanger was not operational, the change to Cl^--free conditions would

not lead to a change in pH_i. This is indeed what appears to happen in the amphibian lens (Wolosin et al., 1989). The experiments carried out in Cl^--free conditions that provided evidence for the bimodal operation of the quadruple carrier are interesting for a quite different reason. Bassnett (1990) found in the chick lens that removing external Cl^- produced a pronounced and prolonged internal alkanization. It was reasoned that Cl^--free conditions would lead to a decrease in cell volume, which would, in turn, stimulate a primarily volume-regulating Na^+-H^+ exchange mechanism, similar to that found in amphibian cells (Wolosin et al., 1989). No such pronounced alkanization was observed in bovine cells, indicating that a volume-sensitive Na^+-H^+ mechanism does not exist in these cells, and this was confirmed by the absence of a significant alkalinization on adding 60 mM sucrose to the bathing medium (*data not shown*).

Many cell types have a sodium-independent, DIDS-sensitive HCO_3^--Cl^- exchange mechanism that serves to acidify the cell interior (Thomas, 1984). In muscle cells this exchanger has been implicated in pH regulation during recovery from internal alkalinization (Vaughan-Jones, 1981). Interestingly, in the present system the removal of Na^+ from the medium also greatly reduces the rate of recovery from the alkalinization induced by CO_2 removal (Fig. 7). It is suggested that in bovine cells, the Na^+-H^+-HCO_3^--Cl^- exchanger operates in the reverse mode under an alkalinizing load to remove HCO_3^- from the cell. Such a system would also be expected to be sensitive to DIDS. In the bovine cells it appears that the simple HCO_3^--Cl^- exchange system is absent as addition of DIDS to a Na^+-free medium produced no effect on pH_i (Fig. 9).

HCO_3^- has a further role to play in lens pH regulation, as its presence leads to a major increase in the measured buffering capacity (Fig. 9). The intrinsic buffering capacity is measured under HCO_3^--free conditions, and the present values of 5 mM/pH unit (procaine) and 8.2 mM/pH unit (NH_4Cl) compare with 16 mM/pH unit obtained in the chick lens epithelium by Bassnett (1990). Interestingly, his larger value was obtained in the presence of both amiloride and Na^+. Hence, although Na^+-H^+ exchange was abolished, the quadruple ion exchanger could still have been operating in the Cl^--Cl^- mode and contributing to the apparent buffering capacity. Thomas (1989) has previously stressed the importance of measuring buffering capacity in the presence of HCO_3^- and, indeed, the bovine data show a four- to fivefold increase compared to the intrinsic value. The contribution to the buffering capacity from the CO_2/HCO_3^- system alone can be estimated from the equation (Roos & Boron, 1981)

$$\beta_{HCO_3^-} = 2.3[HCO_3^-]_i. \quad (2)$$

In the procaine experiments, for example, the mean pH_i during the procaine pulse was 6.83, and hence HCO_3^- is 6.1 mM. This gives a value for β of 14 mM pH, and hence the total theoretical buffering capacity ($\beta_{HCO_3^-} + \beta_{intrinsic}$) is approximately 20 mM/pH. This compares well with the measured value of 27 mM/pH given that the intrinsic buffer capacity is likely to have been underestimated due to lower pH_i in HCO_2/HCO_3^--free media.

The crucial importance of external Na^+ in regulating pH_i can be seen in experiments where Na^+ is substituted by N-methyl-D-glucamine$^+$. Both in the presence and absence of HCO_3^- a rapid acidification occurs, and, interestingly, the final resting pH_i is similar in each case (Figs. 6 and 7). Part of the Na^+ effect arises from inhibiting the quadruple ion exchanger but part must also arise from inhibiting Na^+/H^+ exchange since addition of amiloride (1 mM) invariably reduced pH_i when added either to control or to HCO_3^--free media (Fig. 5). Furthermore, removal of Na^+ at the end of an NH_4Cl pulse totally abolishes the ability of the cell to recover from the acid stress involved (Fig. 8).

Acidification in the presence of external lactate suggests that there is either cotransport of lactate and H^+ or exchange of lactate and OH^- (Mason & Thomas, 1985). Na^+ does not appear to be involved in this transport since addition of lactate to the medium initiated a fall in pH both in the presence (Fig. 10) or absence of Na^+ (*data not shown*). In this respect the lens lactate transporter is similar to that described by Bonanno (1990) in rabbit corneal epithelial cells. However, the responses in the two cell types are different in HCO_3^--free media. In the bovine cells, exposure to lactate induced pronounced transients in the response (Fig. 10*B*), while the responses were monophasic in the corneal cells. In the bovine cells, the responses were also much smaller in the HCO_3^--free media even though the buffering capacity is greatly reduced on removing HCO_3^-. These data indicate the presence of another control mechanism in the absence of HCO_3^-. The alkaline overshoot on removing lactate also indicates that an alkalinizing mechanism has been switched on some time after adding lactate, and that this mechanism is still present after lactate has been removed. Amiloride abolishes this adaptative process, implicating an increase in the rate of Na^+-H^+ exchange in response to the acid stress. Interestingly, a similar magnitude of lactate-induced acid stress in the presence of external HCO_3^- does not lead to an alkaline overshoot.

The modes of operation of the three acid-regulating carrier mechanisms described above are summarized in Fig. 12. Since two of them are dependent

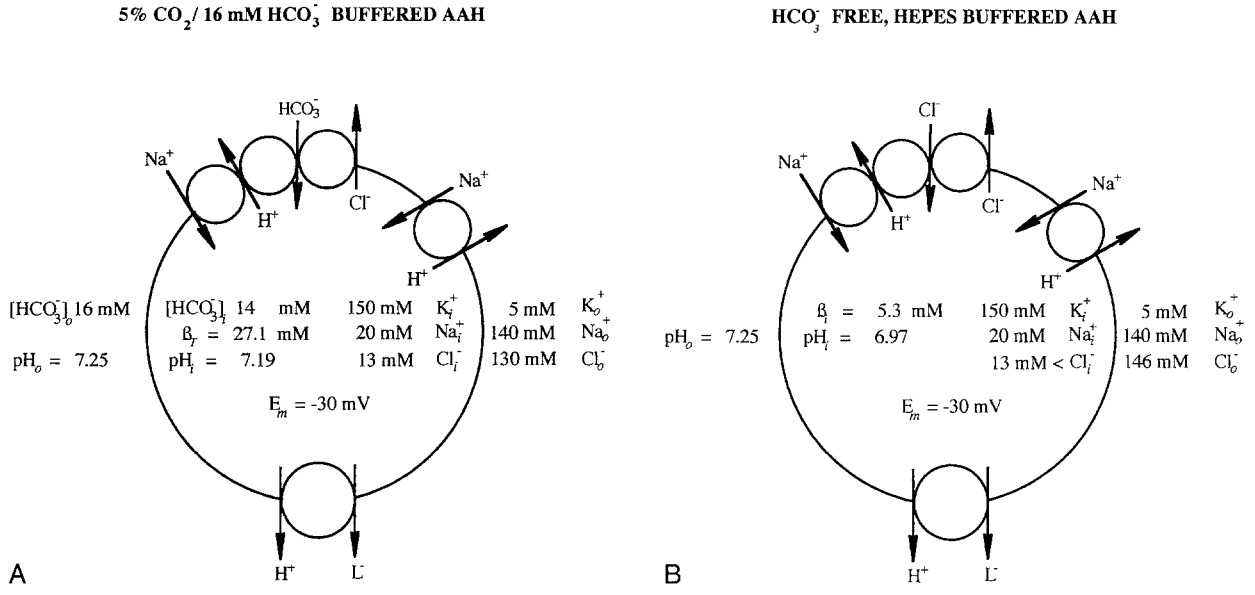

Fig. 12. Summary of acid-regulating systems present in bovine lens cells together with relevant membrane potential and ionic data. The internal concentrations of Na^+, K^+ and Cl^- are whole bovine lenses (recalculated from data given in Duncan & Bushell (1976), while the membrane potential data are taken from Jentsch et al. (1985). The internal bicarbonate concentration in control AAH has been computed assuming pH_i = 7.19 and that internal CO_2 = 5%. No Cl^- values are available for mammalian lens cells incubated in HCO_3^- free media and so the value in *B* has been assumed to be greater than 13 mM.

on the gradients of other ions, previously published values for bovine lens Na^+, K^+ and Cl^- are also given. The data emphasize the considerable electrochemical gradient for Na^+ that is available but highlight the fact that no electrochemical gradient is available to drive the outward movement of Cl^-. In the presence of external HCO_3^-, bicarbonate ions are distributed above equilibrium and hence require to be actively imported. We suggest that the quadruple ion exchanger represents the major mechanism by which this distribution is achieved (*see also* Thomas (1989) for a review). There is no evidence in the present study that HCO_3^- moves across the cell membrane via channels, and, indeed, such a movement would tend to drive HCO_3^- out of the cell down its electrochemical gradient. Such a leak pathway would reduce the pH-regulating power of the quadruple exchanger. Even during the acid phase of the CO_2 transient in bovine cells (Figs. 2 and 7) where HCO_3^- would be expected to be passively leaving the cytoplasm, the efflux appears to be Na^+ dependent and so is unlikely to take place via channels. In a series of electrophysiological experiments, Jentsch et al. (1985) also found no evidence for a HCO_3^- conductance in bovine cultured lens cells.

Figure 12*B* illustrates the operation of the quadruple carrier in the Cl^-/Cl^- exchange mode in the absence of external HCO_3^-, and under these circumstances it is important to note that the exchanger still has a considerable ability to acid regulate pH_i as is demonstrated by the effect of removing external Cl^- in the presence and absence of HCO_3^- (Fig. 4*A* and *B*). It is likely also that the internal Cl^- content of the lens would increase both as a result of an increased inward movement through the quadruple exchanger, and also to maintain electroneutrality. As far as we are aware, all lens chloride values have been determined in the presence of HCO_3^-.

The lactate H^+ cotransporter is the only system that appears to be independent of other ionic gradients, but the fact that considerable time-dependent adaptation and overshoot occur during acid regulation in the absence of HCO_3^- (Fig. 10*B*) indicate that "cross-talk" may well occur among the carriers.

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