Role of Chloride/Bicarbonate Antiport in the Control of Cytosolic pH

CELL-LINE DIFFERENCES IN ACTIVITY AND REGULATION OF ANTIPORT

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Sodium-linked and sodium-independent HCO₃⁻/Cl⁻ antiport was measured under different conditions in a number of cell lines. Transport of HCO₃⁻ was estimated from its effect on intracellular pH (pHi) measured with the fluorescent probe 2',7'-bis(carboxyethyl)-5,6-carboxyfluorescein. The associated ion fluxes were estimated from the transport of ³⁵Cl⁻ and ²²Na⁺. Na⁺-dependent and Na⁺-independent HCO₃⁻/Cl⁻ antiport were found in many, but not in all cell lines tested. The Na⁺-independent HCO₃⁻/Cl⁻ antiport was found to be highly pH-dependant in a number of cell lines, whereas in others this was not the case. Some cell lines were found to have both Na⁺-dependent and Na⁺-independent HCO₃⁻/Cl⁻ antiport, whereas in others we could detect only one of these mechanisms. Na⁺/H⁺ antiport, which is quantitatively the most important H⁺-extruding mechanism, was found in all cell lines tested, but the activity varied strongly. Possible reasons for the qualitative and quantitative differences in antiport activity are discussed.

Strict regulation of the intracellular pH (pHi) is essential for the normal functioning of cells (for review, see Refs. 1–3). The activity of many cytosolic enzymes is strongly pH-dependant. An example is phosphofructokinase, the rate-limiting enzyme in glycolysis, which is activated by a slight increase in internal pH (4). A moderate increase in cytosolic pH is observed when a number of different stimuli induce cells to grow and divide (5–14).

Cells have developed several mechanisms to regulate the internal pH. Among these, the Na⁺/H⁺ exchanger has been studied in most detail (15–17). This antiporter is activated when the internal pH drops below the physiological level. The large inwardly directed Na⁺ gradient is then allowed to extrude protons from the cytoplasm until the pH is normalized and the antiporter is again inactivated.

Na⁺/H⁺ exchange is the main mechanism to extrude H⁺ in the absence of bicarbonate. Work from several laboratories has shown that processes dependent on bicarbonate also play important roles in the control of the internal pH (18–20). We have recently found that in the monkey kidney cells, Vero, bicarbonate is involved in the regulation of pH, both after acidification and alkalization of the cytosol (21–23).

In Vero cells given an acid load the transport of bicarbonate is Na⁺-linked, and the inwardly directed Na⁺ gradient appears to drive bicarbonate (possibly as the ion pair Na⁺/CO₃⁻) into the cells in exchange with Cl⁻. Also Na⁺-independent anion antiport was found to occur in Vero cells and in a number of other cell lines tested, and this antiport was found to be strongly activated at alkaline pH (21, 24, 25). In Vero cells there is approximately a 4–5-fold inwardly directed Cl⁻ gradient under normal conditions. At neutral pH, and at physiological extracellular pH there is only a 2-fold inwardly directed HCO₃⁻ gradient (22). When the pH increases, the intracellular concentration of HCO₃⁻ is also increased (18), and the inwardly directed HCO₃⁻ gradient is therefore further reduced and may even be reversed. Activation of the Na⁺-independent Cl⁻/HCO₃⁻ antiport therefore results in efflux of HCO₃⁻ in exchange with Cl⁻, and, as a consequence, reduction of pH (21, 22). Also, in MDCK cells, Cl⁻/HCO₃⁻ antiport was activated at alkaline pH (26). It should be noted that chloride/bicarbonate antiport is also important for a number of functions other than pH regulation (see "Discussion").

Bicarbonate-dependent pH-regulating processes have been described in a number of cell lines (9, 19, 20, 27–31), while in other cells such processes could not be detected (32). Furthermore, in some cases only Na⁺-dependent HCO₃⁻ transport was observed, (19, 20, 33–39), while in other cases evidence for Na⁺-independent HCO₃⁻/Cl⁻ exchange was obtained (29, 30, 40–43). Since these experiments were carried out in different laboratories and by different techniques, it was not clear if the differences found were due to different experimental approaches. We therefore decided to study and compare under identical conditions the pH regulatory processes in several cell lines. The results showed that different cell lines vary not only with respect to the extent of antiport, but also in the way the antiport is regulated.

EXPERIMENTAL PROCEDURES

Materials—MES, Tris, potassium glutonate, nigericin, Heps, ouabain, choline chloride, choline-HCO₃⁻, and DIDS were obtained from Sigma. Liquid paraffin was from Merck, Darmstadt, FRG. Amiloride was a gift from Merck, Sharpe and Dohme, Darmmen, Norway. BCECF was purchased from Molecular Probes, Eugene, OR. H¹⁴Cl (specific activity 19.6 µCi/mg chloride) and ²⁴NaCl (specific activity 1000 mCi/mg sodium) were from The Radiochemical Centre, Amersham, U.K.

Cells—L-cells, Vero cells, and BHK cells are cell lines that have been growing in our laboratory for years. HEP-2 and BSC-1 cells were obtained from Flow Laboratories, Rickmansworth, U.K.; MDCK cells were obtained from Dr. K. Simons, European Molecular Biology Laboratory, Heidelberg; NIH/3T3 cells were obtained from Dr. T. Ege, Oslo, Norway; HOS, HOS MNN, Calu-1, U-2 OS, and SW 480 were obtained from Dr. G. F. Vande Woode, National Cancer Insti-

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tive to diphtheria toxin, whereas L- and NIH/3T3 cells are resistant to this toxin, but very sensitive to Pseudomonas aeruginosa exotoxin A (45, 46). The other cell lines are moderately sensitive to diphtheria toxin. MDCK cells form "domes" when grown on plastic surfaces, due to their ability to form tight junctions (47). A431 cells have an exceptionally high number of receptors for epidermal growth factor (48).

Upon arrival in the laboratory, the cells were frozen down in aliquots. In the course of this work, new aliquots were thawed at intervals and cultured for subsequent experiments.

Media and Buffers—Hepes medium: Minimum essential medium, except that bicarbonate was replaced by 20 mM Hepes. KCl buffer: 140 mM KCl, 1 mM CaCl₂, and 20 mM MES adjusted to the indicated pH with Tris. K-glucoside buffer: 140 mM potassium gluconate, 1 mM CaCl₂, 20 mM MES adjusted to the indicated pH with Tris. Choline chloride buffer: 140 mM choline chloride, 1 mM CaCl₂, 20 mM MES adjusted to the indicated pH with Tris. PBS (phosphate-buffered saline): 140 mM NaCl and 10 mM sodium phosphate, pH 7.4.

Measurement of ³⁶Cl⁻ Uptake—Cells in 24-well disposable trays were transferred to KCl buffer at the indicated pH for 30 min at 37°C. Then the cells were washed twice with ice-cold potassium gluconate buffer, and 0.3 ml of the same buffer containing 0.2 μCi/ml of ³⁶Cl⁻ was added to each well. After various periods of time at 30°C the cells were washed twice with ice-cold PBS, and then 0.3 ml of 5% trichloroacetic acid was added to each well. After 10 min at room temperature the trichloroacetic acid was transferred to counting vials, and the amount of acid extracted radioactivity was measured.

Measurement of ²²Na⁺ Uptake—Cells in 24-well disposable trays were preincubated in Hepes medium as indicated for 30 min at 37°C. The cells were then washed twice with ice-cold choline chloride buffer. Subsequently, 0.3 ml of the same buffer containing 0.4 μCi/ml of ²²NaCl was added to each well. Ten mM HCO₃⁻ was present when indicated. To prevent escape of ¹⁴C, a layer of liquid paraffin was added to the external buffer (pH). After 30 min preincubation in this buffer, the cells were transferred to K-gluconate buffer, pH 7.0, containing ³⁶Cl⁻, but no nigericin, and the ability of the cells to accumulate ³⁶Cl⁻ was measured.

The data in Fig. 1A show that when HEp-2 cells were preincubated at pH 7.4, the ³⁶Cl⁻ uptake was very rapid. When the preincubation was at pH 6.5, the uptake rate was reduced to approximately 1/10. In both cases the intracellular concentration of ³⁶Cl⁻ reached a level that was 5-10 times higher than that in the buffer. This uphill transport of ³⁶Cl⁻ against its concentration gradient is apparently energized by the efflux of intracellular chloride by anion antipporter (24, 25).

RESULTS

Effect of the Internal pH on ³⁶Cl⁻/Cl⁻ Exchange—In a first set of experiments we tested to what extent the rate of chloride uptake by anion antiporter is regulated by pH, in different cell lines. To adjust the internal pH, the cells were incubated in isotonic KCl buffer containing nigericin, a carboxylic ionophore that carries out H⁺/K⁺ exchange (50). When the concentration of K⁺ is the same at both sides of the membrane, nigericin clamps the pH at the same value as that in the external buffer (pH). After 30 min preincubation in this buffer, the cells were transferred to K-glucoside buffer, pH 7.0, containing ³⁶Cl⁻, but no nigericin, and the ability of the cells to accumulate ³⁶Cl⁻ was measured.

When the uptake of ³⁶Cl⁻ was measured for 2 min in HEp-2 cells preincubated at various pH values, there was an abrupt increase in the uptake rate approximately at pH 7.3 (Fig. 1B). This is in accordance with earlier results from this laboratory (21-25).

In contrast to the findings made with HEp-2 cells and with a number of other cell lines which we have studied earlier (24, 25), the uptake rate of ³⁶Cl⁻ in NIH/3T3 cells was essentially the same at all pH values tested (Fig. 2). The uptake rate in this cell line was close to that found in HEp-2 cells preincubated at low pH (compare Figs. 1A and 2A). Also in NIH/3T3 cells the uptake appears to occur by antipporter. Thus, the...
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Fig. 2. Effect of pH on the uptake of 36Cl− by NIH/3T3 cells. Conditions as in Fig. 1. In B, the uptake was measured after 2, 5, 10, and 20 min as indicated.

Fig. 3. Effect of pH on the uptake of 36Cl− by HOS (●), MDCK (○), Calu-1 (●), and HOS MNNG cells (◇). Conditions as in Fig. 1. In A the pH was 7.4.

Fig. 4. Effect of pH on the uptake of 36Cl− by BSC-1 (●), U-2 OS (○), and SW 480 cells (◇). Conditions as in Fig. 1. In A the pH was 7.4.

Fig. 5. Effect of pH on the uptake of 36Cl− by A431 cells. Conditions as in Fig. 2.

cells accumulated 36Cl− to an internal concentration much higher than that in the medium, and the uptake was strongly inhibited by DIDS (Fig. 2A). It therefore appears that the anion antiport in NIH/3T3 cells is not activated by an increase in the internal pH.

We have also studied the uptake of 36Cl− in a number of other cell lines. Four of these (HOS, HOS MNNG, MDCK, Calu-1) responded to variations in the internal pH in a similar way as HEp-2 (Fig. 3, A and B), although the pH threshold for the abrupt increase in activity, as well as the absolute uptake rate, varied between the different cell lines. This is in accordance with our previous findings with a number of other cell lines (25). In contrast to this, however, three cell lines (SW 480, U-2 OS, BSC-1) did not respond strongly to changes in pH, (Fig. 4), similar to NIH/3T3 (Fig. 2).

Since the 36Cl− uptake measurements were carried out at low extracellular chloride concentration, the possibility existed that the difference in 36Cl− accumulation could reflect difference in leakage of chloride from the cells rather than difference in uptake. To test this, the cells were loaded with 36Cl− and then transferred to K-gluconate buffer containing nigericin and adjusted to different pH values. The radioactivity associated with the cells was measured after increasing periods of time. We found that in most of the cell lines the efflux was comparatively slow at pH < 7.0 (half-time 10–40 min), but it increased between pH 7.0 and 7.5 (data not shown). This is in accordance with our earlier findings with other cell lines (25), and it is probably the reason why the 36Cl− accumulation decreases when pH is increased above an optimal value (see Figs. 3 and 4). The leakage was inhibited by DIDS, but not by bumetanide.

When 10 mM Cl− was present in the K-gluconate buffer, the efflux rate was considerably increased, indicating that the rate of exchange is much higher than that of chloride leakage. The difference was largest at low pH, where the leakage into chloride-free buffer was lowest (data not shown). Clearly, the low 36Cl− uptake at low pH is not due to increased chloride leakage. Furthermore, differences in chloride leakage cannot account for the cell-line differences in 36Cl− accumulation. It may therefore be concluded that both the ability of the antiporter to respond to increased pH and its absolute activity at optimal pH differ strongly between cell lines.

One cell line, A431, was not able to accumulate 36Cl− to a concentration significantly higher than that in the medium at any pH value tested (Fig. 5, A and B). The reason for this is apparently a high chloride permeability in these cells. Thus, in A431 cells, efflux of 36Cl− into K-gluconate occurred at a high rate even at low pH, (data not shown). The efflux rate was not increased by the presence of chloride, supporting the conclusion that measurable exchange does not occur. The efflux was, however, inhibited by DIDS, similar to the efflux occurring at high pH, in the other cell lines studied.

In the experiments presented, the uptake of 36Cl− was measured in the presence of 140 mM gluconate. We have also measured the uptake in buffer osmotically balanced with mannitol and obtained essentially the same results, except that the initial uptake rate was somewhat higher in mannitol than in gluconate (data not shown).

**Effect on the Internal pH of Transfer of Cells to Chloride-free Buffer**—Our earlier data from Vero cells indicated that the antiporter which carries out the chloride self-exchange studied above also carries out HCO3-/Cl− exchange (22, 24).
This activity can be measured when cells kept in KCl-buffer containing HCO₃⁻ are transferred to K-gluconate buffer containing the same concentration of bicarbonate. Under these conditions the outward directed Cl⁻ gradient may induce entry of HCO₃⁻ by antiport, with alkalinization of the cytosol as a result (18, 21, 23, 27). We found that in Vero cells the cytosol was rapidly alkalinized by ~0.3 pH units under these conditions (21).

To further test if the Cl⁻/HCO₃⁻ antiport thus measured is indeed carried out by the same mechanism as the ³⁶Cl⁻/Cl⁻ exchange described above, we tested some of the cell lines analyzed in Figs. 1–5 for increase in pHᵢ upon transfer to K-gluconate buffer. The data in Fig. 6, A, B, and E, show that in HEp-2, MDCK, and HOS cells the transfer resulted in rapid alkalinization that could be inhibited with DIDS. The same cell lines were active in ³⁶Cl⁻/Cl⁻ exchange (see Figs. 1 and 3). Also in NIH/3T3 cells and in BSC-1 cells DIDS-sensitive alkalinization occurred, but in these cell lines it developed at a lower rate (Fig. 6, C and D). These cell lines were also less active in ³⁶Cl⁻/Cl⁻ exchange (Fig. 2 and 4). In A431, where we found no evidence for ³⁶Cl⁻/Cl⁻ exchange (Fig. 6), very little DIDS-sensitive pHᵢ increase was observed upon transfer to gluconate buffer (Fig. 6F). The data indicate that Na⁺-independent Cl⁻/HCO₃⁻ exchange and at least the major part of the ³⁶Cl⁻/Cl⁻ exchange are carried out by the same mechanism and that therefore the results in Figs. 1–5 reflect the activity of the HCO₃⁻/Cl⁻ exchanger.

Ability of Different Cells to Carry Out Bicarbonate-dependent and Sodium-dependent Uptake of ²²Na⁺—In the next series of experiments, we measured the ability of the different cell lines to carry out Na⁺/H⁺ exchange and Na⁺-linked HCO₃⁻/Cl⁻ exchange. Since these processes are most active when the cytosol is acidic (16, 22), we initiated the experiments by giving the cells an acid load. Furthermore, ouabain was present to prevent extrusion of ²²Na⁺ by the Na⁺/K⁺-ATPase.

The acid load was given by the NH₄Cl preloading technique (52). This involves preincubation of the cells in Heps medium containing NH₄Cl to allow NH₄⁺ ions to accumulate in the cytosol. When the cells are subsequently transferred to medium without NH₄Cl, NH₃, which is membrane permeant, rapidly diffuses out from the cells, leaving H⁺ behind in the cytosol. The data in Fig. 7 show that when uptake of ²²Na⁺ was measured under these conditions, all cell lines studied accumulated the isotope to concentrations higher than that in the external buffer. In the absence of HCO₃⁻ the uptake of ²²Na⁺ was in all cases strongly inhibited by 1 mM amiloride, which is an inhibitor of Na⁺/H⁺ exchange (16).

When 10 mM choline-HCO₃⁻ was added, some of the cell lines accumulated ²²Na⁺ even in the presence of amiloride. This was the case in MDCK, BHK, Vero, HOS MNNG, and HEp-2. On the other hand, in MCF-7, Calu-1, HOS, human fibroblasts, A431, and NIH/3T3 there was no evidence for bicarbonate-stimulated uptake of ²²Na⁺. U-2 OS and L-cells represent borderline cases. Clearly there are large variations in the ability of different cells to accumulate ²²Na⁺ in a bicarbonate-dependent manner.

Ability of Different Cell Lines to Carry Out Bicarbonate-dependent and Bicarbonate-independent Regulation of pHᵢ, after an Acid Load—The bicarbonate-dependent uptake of ²²Na⁺ in Vero cells was found to occur as uptake of both Na⁺ and bicarbonate in exchange with Cl⁻ (22). Since entry of bicarbonate increases the internal pH, we carried out experiments where we measured the ability of the different cell lines to extrude H⁺ equivalents by anion antiport. We also carried out experiments in the absence of bicarbonate to assess the Na⁺/H⁺ antiport activity.

Also in these experiments we acidified the cytosol by the NH₄Cl-prepulsing method. Cells were incubated for 5 min in Heps medium at pH 7.6 containing 15 mM NH₄Cl to allow NH₄⁺ to accumulate in the cells. Choline bicarbonate (10 mM) was present when not otherwise indicated. Upon subsequent removal of NH₄⁺ from the medium, the cytosol was acidified to pHᵢ values between 6.2 and 6.5.

The data in Fig. 8 show that in the absence of amiloride in all cell lines tested the internal pH started to increase immediately after the acidification. Amiloride reduced the rate of pHᵢ regulation in all cells tested. In BHK and HEp-2 cells the pHᵢ regulation was further reduced when DIDS was present or when no bicarbonate was added (Fig. 8, A and B). On the other hand, in NIH/3T3 and A431 cells HCO₃⁻ and DIDS did not influence much the rate of pHᵢ increase (Fig. 8, C and D). The results are in accordance with the data in Fig. 7 showing bicarbonate-dependent uptake of ²²Na⁺ in BHK and HEp-2 cells, but not in NIH/3T3 and A431 cells. Altogether, the results indicate that in HEp-2 and BHK cells the regulation of pHᵢ after an acid load is due both to Na⁺/H⁺ antiport (inhibited by amiloride) and to anion antiport (inhibited by DIDS), while in NIH/3T3 and A431 the pHᵢ recovery occurs mainly by Na⁺/H⁺ exchange.

Quantitative Measurements of the Transport Activities in Different Cell Lines—In the experiments shown in Fig. 7, the cells were grown to confluence. Since the cell density at confluence differed considerably from one cell line to another, the difference in ²²Na⁺ uptake as here measured cannot be used to compare the antiport activity between different cell lines.

To obtain a more comparable measure, we estimated the rate of transport from the number of H⁺ equivalents extruded from the cells under the different conditions. To carry out these calculations, it was first necessary to determine the volume of the cells and the intrinsic buffering capacity (δᵢ) of the cytosol. To measure δᵢ, we determined the change in pHᵢ upon addition and removal of NH₄Cl from the medium as
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**FIG. 7.** Bicarbonate-independent and bicarbonate-dependent uptake of $^{22}\text{Na}^+$ in different cell lines. Cells in 24-well disposable trays were incubated for 30 min at 37 °C in Hepes medium, pH 7.4, containing 30 mM NH$_4$Cl and 50 μM ouabain. When indicated, 1 mM amiloride was present for the last 5 min of the preincubation. The cells were washed, and choline chloride buffer, pH 7.4, containing 50 μM ouabain and 0.4 μCi/ml $^{22}\text{Na}^+$ was added. When indicated, 1 mM amiloride and 10 mM HCO$_3^-$ were also present in the buffer. Liquid paraffin was added to avoid escape of CO$_2$. After increasing periods of time, the radioactivity associated with the cells was measured. The radioactivity of the external buffer was ~800 cpm/μl and the total cell volume in one well varied between 0.2 and 0.7 μl. A, no amiloride or bicarbonate added; □, 1 mM amiloride, no bicarbonate added; ○, 1 mM amiloride and 10 mM choline bicarbonate added.

**FIG. 8.** Regulation of pH$_i$ after acidification of the cytosol. Cells loaded with BCECF were preincubated for 5 min with 15 mM NH$_4$Cl in Hepes medium, pH 7.6, with 10 mM choline bicarbonate when not otherwise indicated. Then the cells were transferred to NH$_4$Cl-free Hepes medium containing 10 mM choline bicarbonate when not otherwise indicated. Amiloride (1 mM) and DIDS (0.1 mM) were present when indicated. The fluorescence was recorded. The cells used were: A, BHK; B, HEp-2; C, NIH/3T3; D, A431.
cell lines differ widely in the rate at which the three pH,
regulatory mechanisms studied here are operating. All cell
lines examined appear to possess the Na+/H+ exchanger, in
accordance with the data in the literature (for review, see Ref.
2). Thus, to our knowledge, only a cell line that was selected
for the absence of the Na+/H+ exchanger (19, 34, 53) was
found to lack Na+/H+ exchange activity. We here found that
the rate of exchange differs strongly between different cell
lines. Part of this difference could, however, be due to different
pH sensitivity of the Na+/H+ antiporter (16).
Large differences were also found with respect to anion
antiport. Both 36Cl/Cl− exchange experiments and measure-
ments of pH; upon transfer of cells to Cl−-free, HCO3−-con-
taining medium showed that the Na+-independent anion
exchange occurred at a high rate in Hep-2, MDCK, Calu-1,
HOS, and HOS MNGG cells at alkaline pH, while the rate
was much lower when the internal pH was low. This is similar
to our previous findings with Vero cells (21–25). In contrast,
in several other cell lines (NIH/3T3, BSC-1, U-2 OS, SW
480) the rate of this antiport did not change much with
variations in pH. The finding that those cell lines that were
most active in 36Cl−/Cl− exchange were also most active in
Cl−/HCO3− exchange, supports our earlier conclusion (22, 24,
25) that these two functions are carried out by the same
antiporter.
In one cell line (A431), we did not find evidence for 36Cl−/
Cl− antiport at all. This could be due to short-circuiting of
the antiport by the high chloride leakage in these cells.
In those cases where we have measured the intracellular
chloride concentration, it was found to be in the range 20–40
mM (25). It can be calculated from the intrinsic buffering
capacity (β) of the cells (Table II) that exchange of all
intracellular chloride with HCO3− should increase the internal
pH by 1–2 pH units. In contrast to this, we found (Fig. 6) that
pH increased only by 0.2–0.3 pH units. Also, other
authors (30, 54) have found a pH increase of this magnitude
under similar conditions. We think that the reason for this
discrepancy is that at the increased pH, a chloride conduct-
ance is activated (25). Leakage of chloride from the cells
would then short-circuit the antiport.
The different cell lines also differ strongly with respect to
the rate of Na+-linked uptake of bicarbonate. In some cases
(MDCK, BHK, Hep-2, L), this mechanism contributed con-
siderably to the normalization of pH, after an acid load, while
in other cell lines (e.g. HOS, A431, NIH/3T3) it was insignif-
ificant. It should be noted that in some cell lines (e.g. HOS,
bicarbonate-linked uptake of Na+ could be detected. This indicates that the two kinds of anion antiport are carried out by different molecules.

The data presented in Table III probably represent underestimations of the role of the bicarbonate-dependent pH regulation. For technical reasons (to better control the pH in the medium) the experiments were carried out in the presence of only 10 mM HCO3−, whereas the physiological concentration is approximately 3 times higher. It should also be noted that in those cells where we did not observe Na+-linked bicarbonate/chloride exchange, we do not claim that it is totally absent. Our main conclusion is that there are large variations between cell lines with respect to the extent to which the different antiports are expressed.

Our results with A431 cells differ from data previously published by other authors (9, 55) indicating that A431 cells have active Na+-linked HCO3−/Cl− exchange. The reason for this discrepancy is not clear, but it could be due to different strains of A431 cells. We have established that the A431 cells used in our work have a high number of receptors for epidermal growth factor (~106/cell) which is a marker for this cell line.

Similar cell-line differences in pH regulation as those here observed on cells in culture also appears to occur in situ. Thus, Paradiso et al. (56) reported recently considerable differences between the pH regulatory mechanisms in two adjacent cell types in the gastric mucosa. They presented evidence that in oxyntic cells basolateral HCO3−/Cl− antiport is involved in correcting the alkalinization of the cytosol that occurs upon apical extrusion of H+ by the K+/H+ ATPase. On the other hand, in the adjacent chief cells there was no evidence for HCO3−/Cl− exchange, and Na+-dependent processes were found to be active in increasing the internal pH to correct for the constant influx of H+.

Physiological Roles of Anion Antiport in Different Cells—The most obvious role of the antiports here described is to regulate pH. It is well established that the Na+/H+ antiport is activated when pH drops below the normal value and that it is turned off again when the correct pH has been reestablished (67, 58). However, a cell line that lacks the Na+/H+ antiporter was found to be able to regulate the pH; to a limited extent in HCO3−-containing medium (19, 24), and this was also the case in other cell lines when the Na+/H+ exchange was blocked with amiloride or related compounds (9, 29, 37, 55). Under these conditions, Na+-linked HCO3-/Cl− exchange may increase the pH; after acidification, whereas the Na+-independent HCO3−/Cl− exchange may reduce the pH; upon alkalinization. In many cells the Na+-independent HCO3−/Cl− antiport is regulated by the pH; but in the opposite way of that described for Na+/H+ antiport, which increases in activity with decreasing pH; (57, 58).

The scheme in Fig. 9a indicates how we think the HCO3−-dependent regulation of pH; occurs. Because the inwardly directed Na+ gradient is usually larger than the inwardly directed Cl− gradient, the Na+-linked antiporter (here indicated as a NaCO3−/Cl− exchanger)9 carries Na+ and carbonate into the cells. The carbonate may bind 2 protons and thereby increase the pH;. The Na+ that enters together with the carbonate is extruded by the Na+,K+-ATPase. When a critical pH; level is reached, the Na+-independent HCO3−/Cl− exchanger is activated. At pH; above neutrality the inwardly directed HCO3− gradient is smaller than the inwardly directed Cl− gradient, and internal HCO3− is therefore exchanged with external Cl−. As a result, the pH; is reduced. It is likely that the antiporters here discussed also play important roles in processes other than pH; regulation. Some possible functions will be discussed briefly.

In the pancreas, extensive excretion of NaHCO3 takes place. The primary secretion in the acini resembles serum in ionic composition. On its way down the ductuli, particularly after stimulation with secretin, the larger part of the Cl− is exchanged with HCO3−. This exchange is inhibited by ouabain and SITS, but not by amiloride (for review, see Ref. 61). Similar, albeit less extensive, transport of bicarbonate may occur across the surface epithelium cells in the stomach to protect the cells against the gastric acid.

In the early proximal convoluted tubules of the kidney, acidification of the urine takes place by proton excretion and reabsorption of carbonic acid, apparently by the carbonic anhydrase-rich cells (63, 64). It is generally accepted that the alkalinization of the cytosol that occurs as a consequence is compensated for by efflux of bicarbonate at the basolateral side, either, as HCO3−/Cl− exchange (31, 40, 65) or as Na+- linked bicarbonate transport (37, 38, 43, 66–68).

It is also possible that antiport is involved in NaCl reabsorption in the kidney. At the apical (luminal) side of the brush border, Na+/H+ exchange has been demonstrated by many authors (15, 69, 70), and HCO3−/Cl− exchange has also been found on the apical side (71–73). NaCl absorption may therefore partly occur as a parallel exchange by these two antiporters (71). In this way H2CO3 would act merely as a catalyst. Such parallel exchange may also be involved in salt absorption in other organs, such as salivary glands, sweat glands, and in the gut. Parallel antiport also appears to carry salt (and, as a consequence, obliged water) into shrunken Necturus gall bladder (35, 74–76) and lymphocytes (77, 78). The Na+ may be extruded in exchange with K+ by the Na+,K+-ATPase (Fig. 9b). It should be noted that, although exchan-
gers have lower capacity than ion channels, their high number may compensate for the lower activity.

Parallel transport by the two antiporters may also be involved in the stimulation of cell growth by a number of compounds and conditions that activate the Na+/H+ exchanger. Most of these studies have been carried out in nominally bicarbonate-free medium, and an increase in pH has regularly been observed (5-14). However, in those cases where the experiments were carried out in the presence of bicarbonate, there was little increase in the pH, and instead an increase in cell volume was found (55, 57). This is the effect expected if the increased pH activates the HCO₃⁻/Cl⁻ exchanger with the net effect that NaCl accumulates in the cytoplasm with entry of H₂O and cell swelling as the result. This volume increase, rather than the pH increase, could be the signal to induce cell growth and division.

It should be noted that even in the absence of the Na+/H⁺ exchanger and in experiments where it was blocked by amiloride or related compounds, cell growth could be initiated in bicarbonate-containing medium (13, 19, 79, 80). Fig. 9c indicates how the Na⁺-dependent and the Na⁺-independent antiporters alone may increase the cellular Na⁺ content and induce cell swelling. In this model, Na⁺CO₃ brings 2 OH⁻ equivalents into the cell in exchange with a single Cl⁻. The alkanilization of the cytosol is corrected by 2 cycles of HCO₃⁻/Cl⁻ exchange. The net effect is entry of 1 molecule of NaCl.

In many cells the chloride activity appears to be close to the electrochemical equilibrium (81, 82), but in other cases the internal chloride activity has been found to be above (27, 41, 83) or below (84) this equilibrium. The chloride activity of the cytosol may also vary with the regulatory state of the cells (85). The activity of the antiporters described here could account for such differences. Activation of the Na⁺-independent anion antiporter would tend to increase the internal Cl⁻ concentration at the expense of HCO₃⁻. This would result in reduction of the pH. If the Na⁺/H⁺ antiporter is activated and, as a consequence the pH increases again, the net result will be that NaCl enters the cells. This would result in cell swelling as in Fig. 9b.

Although the major part of the intracellular anions are impermanent macromolecules, cell swelling must result in a higher proportion of permeant anions in the cells, primarily HCO₃⁻ and Cl⁻. If the pH remains constant, the intracellular Cl⁻ concentration must be increased. If the membrane potential is not altered in this process, and if chloride-equilibrating mechanisms such as Na⁺,K⁺,2Cl⁻ cotransport or Cl⁻ channels are not operating, intracellular chloride could reach levels considerably above its electrochemical equilibrium. The driving force would again be the Na⁺,K⁺-ATPase that maintains the inwardly directed Na⁺ gradient.

Also values of Cl⁻ below the electrochemical equilibrium could be obtained by appropriate activation of the antiporters. Thus, the inwardly directed Na⁺ gradient could drive Cl⁻ out of the cells via the Na⁺-dependent chloride/bicarbonate exchanger. In this case the alkanilization of the cytosol obtained as a consequence would have to be regulated by other mechanisms than HCO₃⁻/Cl⁻ exchange (Fig. 9d). Conceivably, influx of H⁺ down its electrochemical gradient could maintain the correct pH under these conditions.

So far, the anion antiporters in nucleated cells have not been identified. In erythrocytes, band 3 carries out HCO₃⁻/Cl⁻ exchange (51). The gene for band 3 in mouse erythrocytes has been cloned and sequenced (86), and the gene has also been shown to be expressed in mouse kidney (87). A number of closely related genes have been identified (87-90), but it is not clear in which tissues and to what extent they are expressed. Taking into account the many different functions anion antiport might fulfill in different tissues, and the different stimuli it may have to respond to, it would not be surprising if a family of different, although closely related, antiporter molecules will be identified.

REFERENCES

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