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UNIQUE PERMEABILITY BARRIER OF THE APICAL SURFACE OF PARIETAL AND CHIEF CELLS IN ISOLATED PERFUSED GASTRIC GLANDS

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Summary

Although many factors can influence intracellular pH (pHi), some of the most important are those that involve the movement of acids and bases across the cell membrane. We will discuss recent results concerning barriers to the movement of H^+, NH_3 and CO_2 across the apical cell membranes of gastric gland cells. Cell membranes are generally highly permeable to small, lipophilic molecules such as NH_3 and CO_2. In fact, only two examples are known of membranes relatively impermeable to NH_3 and none of membranes permeable to CO_2. We recently developed a technique for perfusing the lumen of a single hand-dissected gastric gland on the stage of a microscope, while monitoring pHi with a fluorescent dye. We observed the expected pHi changes when we exposed the basolateral (i.e. blood-side) membrane to a pH 6.4 solution (a large, rapid pHi decrease), to a pH 7.4 solution containing approximately 0.3 mmol l^{-1} NH_3 (a large and rapid pHi increase) or to a pH 7.4 solution equilibrated with 1 % CO_2 (a rapid pHi decrease of −0.08). However, pHi was not significantly affected by perfusing the lumen with a pH 1.4 solution, with a pH 7.4 solution containing as much as 2.7 mmol l^{-1} NH_3 or with a pH 6.1 solution equilibrated with 100 % CO_2. These data indicate that a barrier at or near the apical membrane has a uniquely low permeability to H^+, NH_3 and CO_2.

Introduction

One of the more remarkable adaptations in biology is seen in the stomach, which on the one hand secretes an extremely acidic fluid rich in the proteolytic enzyme pepsin and on the other has evolved mechanisms for protecting from this lethal mixture the epithelial cells that line the stomach. Two mechanisms of this protection have been clear for some time. One is the rapid turnover of gastric epithelial cells, which are replaced every 2–4 days (McDonald *et al.* 1964). The second is a coating of mucus and a film of HCO_3^--rich fluid that is sandwiched between the mucus and the apical (lumen-facing) surface of the epithelial cells (see Silen, 1985). However, as shown in Fig. 1A, this mucus–HCO_3^- barrier is believed to cover only the cells on the surface of the stomach, as well as those in the more superficial regions of the gastric glands. There is no obvious mucus barrier

Key words: stomach, pH, fluorescent dyes, acid, carbon dioxide, ammonia.

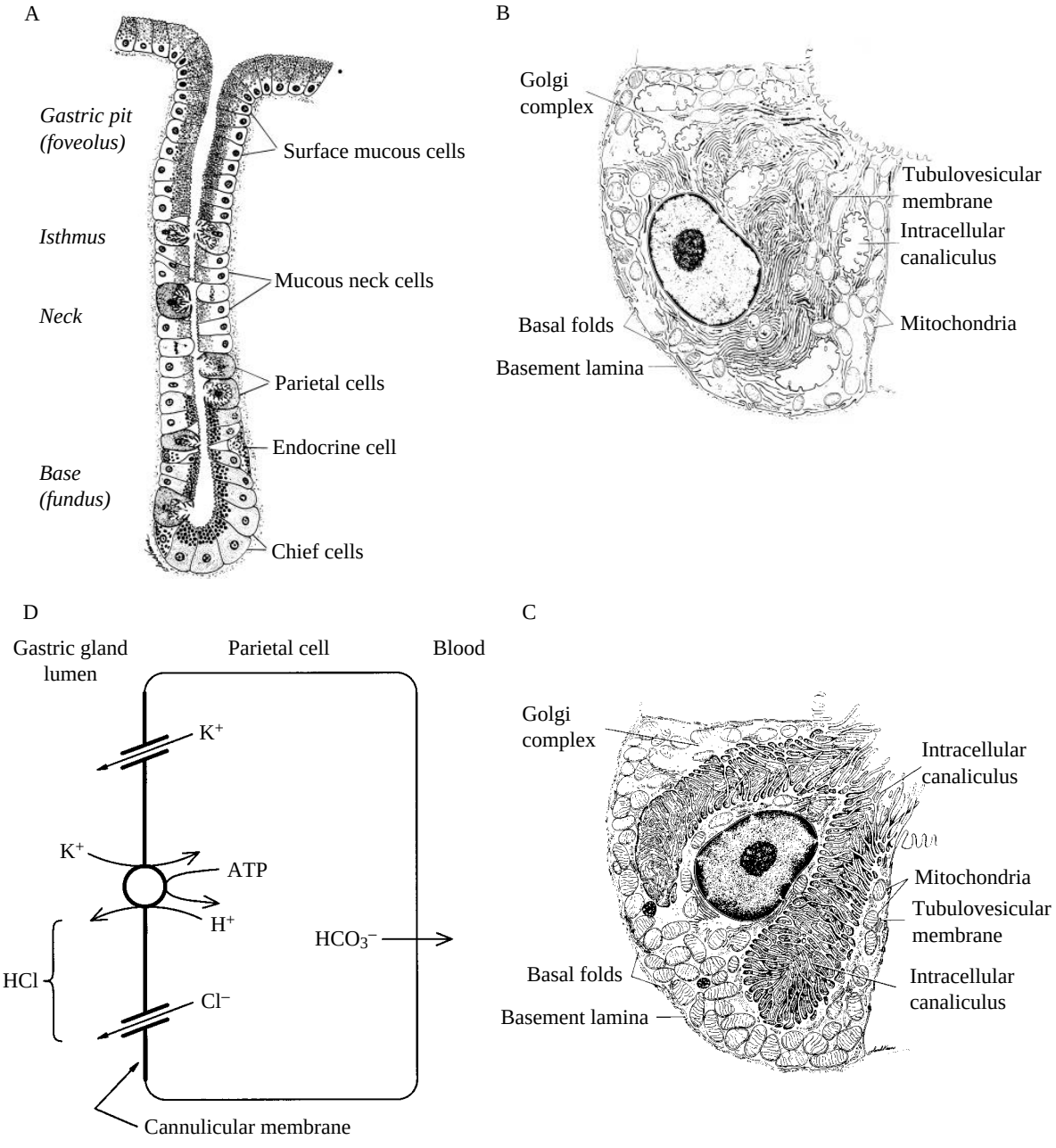

Fig. 1. The gastric gland and mechanism of gastric acid secretion. (A) A schematic diagram of the gastric pit and gastric gland from the major portion of the fundus of the stomach. The gland, which is also known as an oxyntic gland, contains mucous neck cells (which were not studied in our work), the acid-secreting parietal cells, the pepsinogen-secreting chief cells and endocrine cells. (B) A drawing of the unstimulated, acid-secreting parietal cell. (C) A drawing of the stimulated parietal cell. (D) Ionic model of gastric acid secretion by the parietal cell. A–C are reproduced from (Ito, 1987), with the permission of Raven Press.

covering the apical membranes of the parietal and chief cells deeper in the gastric gland (Waisbren *et al.* 1994*a*); these cells are responsible for secreting HCl and pepsinogen, respectively. This raises the question of how these cells are protected from the powerful digestive agents they secrete.

Intuition suggests that, at least for the acid-secreting parietal cell, the protective barrier must be at or extremely close to the apical membrane. Gastric acid secretion is generally believed to be the responsibility of three transport molecules located in the cannulicular membranes that represent an amplification of the parietal cell's apical membrane (Fig. 1B,C). The first is the H^+/K^+ pump (Sachs, 1987), which is homologous to the ubiquitous Na^+/K^+ pump (DiBona *et al.* 1979) and fueled by the hydrolysis of ATP. The H^+/K^+ pump is a well-characterized electroneutral transporter that moves H^+ from the cytoplasm to the gastric gland lumen in exchange for K^+ (Fig. 1D). The second key transport molecule is the K^+ channel that allows the efflux (i.e. recycling) of K^+ across the cannulicular membrane, thereby providing sufficient luminal K^+ to support the action of the H^+/K^+ pump (see Forte and Wolosin, 1987). The third key transport molecule in the cannulicular membrane is believed to be a Cl^- channel that mediates the efflux of Cl^- (Reenstra and Forte, 1990). Thus, the net effect of these three components would be the movement of HCl from the cytoplasm into the lumen of the gastric gland. The pH in the lumen of the stomach can reach values below 1.0 (Nordgren, 1963), and therefore the pH at the external surface of the cannulicular membrane must be at least this low. If the secreted H^+ is to have access to the lumen, and if the K^+ is to have access to the external surface of the H^+/K^+ pump, then one would think that the protective barrier would not cover the pump. However, if the pH 1 fluid secreted by the pump is not to digest the cannulicular membrane, then it is reasonable to suppose that the protective barrier extends to the perimeter of the pump and channels.

Until recently, studying the barrier properties of gastric parietal and chief cells has been extremely difficult. Because the permeability of the apical membranes to acid is expected to be orders of magnitude less than the permeability of the basolateral (blood-facing) membranes, it would be impossible to assess the relative permeabilities of the apical and basolateral membranes in isolated cells: large amounts of acid would traverse the basolateral membrane, regardless of the permeability properties of the apical membrane. Similarly, it would be difficult to draw conclusions from purified apical membranes, inasmuch as even slight contamination would render the preparation far leakier than the intact apical membrane. One way around these problems is to isolate a single gastric gland and to perfuse its lumen. In this article, we describe our recent introduction of the isolated, perfused gastric gland preparation (Waisbren *et al.* 1994*a*) and how this can be exploited to assess the unusual permeability properties of the gastric gland apical membranes (Waisbren *et al.* 1994*a*,*b*).

Materials and methods

Isolation and perfusion of single gastric glands

Although large numbers of glands can be isolated by enzymatic digestion (Paradiso *et al.* 1987), we found that glands isolated by such a method invariably leaked or even lost

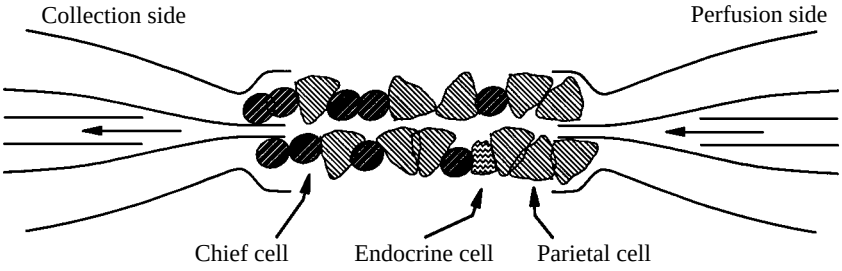

Fig. 2. System for perfusing an isolating gastric gland.

cells during luminal perfusion. Therefore, we resorted to a technique introduced by Schettino *et al.* (1985), dissecting single glands by hand. After transferring the gland to a chamber on the stage of an inverted fluorescence microscope, we cannulated the lumen using an approach originally introduced for the perfusion of single renal tubules (Burg *et al.* 1966). As illustrated in Fig. 2, a system of three concentric pipettes is used to cannulate the blind, distal end of the gland. The outermost ('holding') pipette surrounds the gland. The middle ('perfusion') pipette is used first to puncture the blind end of the gland and then to cannulate the lumen and deliver the perfusate. The innermost ('exchange') pipette is used to deliver the luminal perfusate to the perfusion pipette. Because the vast majority of the solution flowing out of the exchange pipette flows to waste *via* a drain, and only a tiny fraction actually perfuses the gland lumen, it is possible to make relatively rapid changes in the composition of the luminal perfusate. The open, proximal end of the gland is cannulated by a similar set of concentric pipettes.

Because the gland lumen is narrow (diameter <10 μm *versus* approximately 20 μm for the renal proximal tubule) and highly tortuous, the actual perfusion of the gland lumen is rather difficult. To produce an acceptable rate of flow through the gland lumen, we elevated the reservoirs holding the luminal perfusion solutions to approximately 50 cm above the chamber and applied an additional 10–20 kPa of pressure. We verified luminal perfusion at the beginning and end of each experiment by visualizing the color change caused by switching the luminal perfusate from a clear saline solution to one containing Trypan Blue. All experiments were carried out at 37 °C.

The use of digital imaging to monitor intracellular pH

We measured intracellular pH by loading the parietal and chief cells with the fluorescent pH-sensitive dye BCECF (Rink *et al.* 1982). A system of filters and dichroic mirrors (Boyarsky *et al.* 1988) delivered a beam of excitation light, *via* the epi-illumination system of the microscope, switching between wavelengths of 440 and 490 nm. The emitted fluorescent light, filtered at 530 nm, was amplified by an image intensifier and detected by a CCD-type TV camera. Every 8 s, an image-analysis system with an 8-bit analog-to-digital converter grabbed a pair of 480×512 pixel frames, one while exciting the dye at 440 nm and the other while exciting at 490 nm. The emitted intensity is highly sensitive to changes in pHi when the BCECF is excited at 490 nm, but nearly pH-insensitive when excited at 440 nm. The ratio of intensities obtained at 490 and 400 nm is thus an excellent measure of pHi, but is relatively insensitive to such variables

as dye concentration and focus. The digitized images are stored on hard disk and then on a digital tape for later analysis. We used a custom-built software package to draw perimeters around cells and to compute the average intensity of all pixels represented by that cell. At the end of each experiment, we calibrated the intracellular dye by exposing the basolateral surface of the gland to a Na^+-free, high-K^+ nigericin-containing solution at pH 7.00 (Thomas *et al.* 1979; Boyarsky *et al.* 1988). The data obtained under these conditions were used to compute pHi values for each of several cells over the course of the entire experiment.

Results

Effect of low-pH apical and basolateral solutions on pHi

Fig. 3 shows a pHi record obtained from a parietal cell in an experiment in which we examined the effects of either perfusing the lumen or superfusing the basolateral surface of a gastric gland with a low-pH solution. Because other experiments showed that perfusing the lumen with either a pH 6.4 or a pH 3.4 solution had no effect on the pHi of either parietal or chief cells, in this experiment we examined the effect of a pH 1.4 solution in the lumen. As can be seen, no significant pHi change occurred either when the pH 1.4 solution was applied or when it was withdrawn. In contrast, when we briefly exposed the basolateral surface of the gland to a pH 6.4 Hepes-buffered solution, pHi fell rapidly and by a substantial amount. We made similar observations with chief cells, and with both parietal and chief cells in glands that had been stimulated with the acetylcholine agonist carbachol.

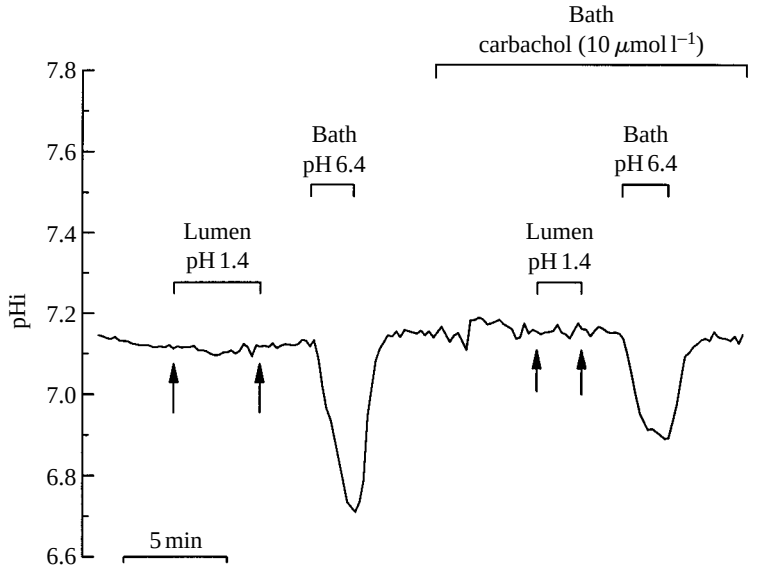

Fig. 3. Effect on parietal cell pH of acidifying either the apical or basolateral surface of the gland. Similar results were obtained on chief cells from the same gland. Reproduced from Waisbren *et al.* (1994*a*), with the permission of the American Physiological Society.

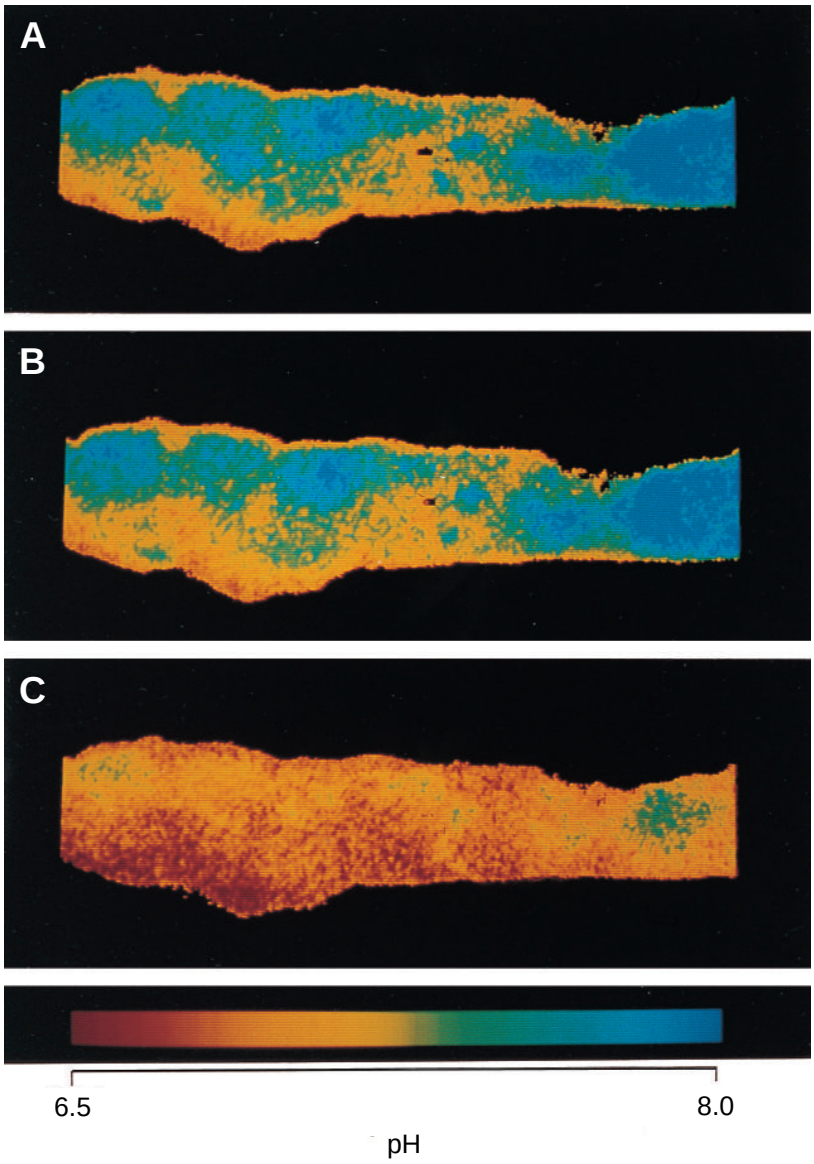

Fig. 4. False-color pHi images of an isolated perfused gland exposed from either the apical or basolateral surface to acidic solutions. The gland was subjected to the same protocol shown in Fig. 3. (A) Control conditions. The gland was exposed to a Hepes-buffered pH 7.4 solution from both the apical and basolateral surfaces. (B) Luminal acidification. The pH of the luminal perfusate was reduced to 1.4. The basolateral solution was the usual Hepes-buffered pH 7.4 saline. (C) Basolateral acidification. The pH of the basolateral perfusate was reduced to 6.4, using a Hepes buffer. The apical solution was the usual Hepes-buffered pH 7.4 saline.

Fig. 4 shows three false-color images of an isolated perfused gland subjected to the same protocol shown in Fig. 3. As shown in Fig. 4A, pHi is relatively uniform throughout except for some low-pHi regions along the center of the gland. In some images (see Fig. 7), these areas of low pHi more noticeably follow a spiral pattern down the length of the gland. It is possible that this spiral may represent the parietal cells, which have been reported to be arranged in such a pattern. Indeed, we have found that the average pHi of parietal cells (approximately 7.21) is somewhat lower than that of chief cells (approximately 7.27). The middle image in Fig. 4B shows that decreasing the luminal pH to 1.4 has a negligible effect on pHi throughout the gland. However, as shown in Fig. 4C, decreasing basolateral pH only from 7.4 to 6.4 causes a substantial intracellular acidification.

Our experiments do not address the mechanism by which acid crosses the basolateral membrane. Work by Paradiso and Machen indicates that parietal and chief cells have both Na^+/H^+ exchangers (Paradiso and Machen, 1987) and Cl^-/HCO_3^- exchangers (Paradiso *et al.* 1987; Seidler *et al.* 1989) at their basolateral membrane, and that parietal cells have a Na^+/HCO_3^- cotransporter at the basolateral membrane (Paradiso *et al.* 1989; Curci *et al.* 1987). Thus, all transporters thus far described at the basolateral membranes of either parietal or chief cells are linked to Na^+ or Cl^-. Indeed, Machen and Paradiso refer to experiments in which they found that altering basolateral pH in non-perfused gastric glands has no effect on pHi if the extracellular fluid lacks both Na^+ and Cl^- (Machen and Paradiso, 1987). Our observation that a million-fold increase in $[H^+]$ in the lumen has a negligible effect on pHi, whereas a mere tenfold increase in $[H^+]$ in the bath produces a substantial pHi decrease, indicates that the barrier to acid movement at the side of the cell is far greater than at the basolateral membrane.

Effect of NH_3-containing solutions on pHi

For two reasons, it is perhaps not too surprising that the apical border of both the parietal and chief cells should have an immeasurably low permeability to H^+. In the first place, from a teleological point of view, both cell types must have an extremely low H^+ permeability at their apical barriers in order to survive in the extraordinarily acidic environment that exists during gastric acid secretion. Second, from a membrane-physiology point of view, it is perhaps not difficult to imagine a membrane constructed in such a way that both the lipids on the outer leaflet and the proteins embedded in the membrane are unusually resistant to the movement of H^+ in the lumen-to-cytoplasm direction. However, it is more difficult to imagine membranes with low permeabilities to lipid-soluble solutes. Indeed, an early tenet of membrane physiology was that neutral lipophilic molecules are far more permeant than their charged, hydrophilic conjugate weak acids or bases. In experiments in which they measured rates of pHi decrease produced by exposing cardiac Purkinje cells to various neutral weak acids, De Hemptinne *et al.* (1983) found that permeability increased as expected with increases in the oil:water partition coefficient. The only exception was for the relatively hydrophilic lactic acid, which is transported across the plasma membrane on a carrier.

In this light, the observations that membranes of two cells have an unusually low permeability to NH_3 are especially surprising. Garvin and Knepper (Garvin *et al.* 1988),

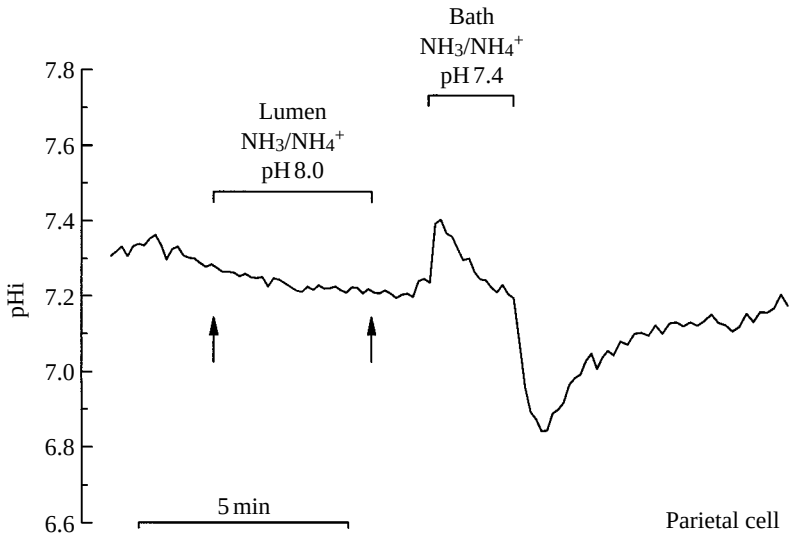

Fig. 5. Effect on parietal cell pH of introducing NH_3/NH_4^+ solutions to either the apical or basolateral surface of the gland. Similar results were obtained on chief cells from the same gland. Reproduced from (Waisbren *et al.* 1994*b*), with the permission of Macmillan Magazines, Ltd.

studying NH_3/NH_4^+ transport, as well as Hebert and his colleagues (Kikeri *et al.* 1989), who monitored pHi changes, showed that the apical membrane in one segment of the renal tubule (the thick ascending limb of Henle's loop) must have an extremely low permeability to NH_3. Later, Frömter and his colleagues, monitoring pHi in *Xenopus* oocytes, showed that the plasma membranes of these cells have a very low permeability to NH_3.

With these observations in mind, we compared the effects on pHi of presenting a solution containing NH_3/NH_4^+ to either the apical or basolateral surface of a perfused gastric gland. Fig. 5 is a pHi record from a single parietal cell in an experiment on an isolated perfused gland. At the indicated time, we changed the luminal solution to one in which 20 mmol l^{-1} NaCl was replaced with 20 mmol l^{-1} NH_4Cl; simultaneously, we increased luminal pH from 7.4 to 8.0. For this total NH_3/NH_4^+ concentration and pH, we calculate that the luminal NH_3 concentration ($[NH_3]_l$) was 1.46 mmol l^{-1}. If the apical barrier of the parietal cell were permeable to NH_3, we would expect the influx of NH_3, and its subsequent protonation to form NH_4^+, to lead to an increase in pHi. Given the rather high extracellular NH_3 concentration and a reasonable intracellular buffering power, we would have expected pHi to increase by several tenths of a pH unit. However, we observed no measurable change in pHi in either parietal cells or chief cells.

In the second part of the experiment shown in Fig. 5, we replaced 20 mmol l^{-1} NaCl in the bath with 20 mmol l^{-1} NH_4Cl. In this case, however, bath pH remained at 7.4. Thus, basolateral $[NH_3]$ ($[NH_3]_b$) was 0.40 mmol l^{-1}, about one-quarter the level of NH_3 we had previously introduced into the lumen. Nevertheless, this lower level of basolateral NH_3 led to a prompt intracellular alkalization. The remainder of the experiment illustrates

the pHi record of a rather typical NH_4^+ prepulse, as seen in a wide variety of cells (see Boron and De Weer, 1976; Roos and Boron, 1981). The slow, plateau-phase pHi decrease that follows the initial increase is due to the influx of NH_4^+ as well as to other acid-loading processes. When the basolateral NH_3/NH_4^+ is washed out, intracellular NH_4^+ dissociates into NH_3 (which rapidly leaves the cell) and H^+ (which is trapped inside). Thus, pHi rapidly decreases. However, this intracellular acid load is followed by a slower pHi recovery that probably reflects the activity of an acid-extruding mechanism, such as a basolateral Na^+/H^+ exchanger. We observed similar pHi transients in chief cells exposed to basolateral NH_3/NH_4^+.

These results suggest that the apical barrier of parietal and chief cells may have an extremely low permeability to NH_3. However, considering only the evidence presented thus far, one might raise several objections to this conclusion. First, perhaps exposing the apical membranes of parietal and chief cells to a solution containing 1.46 mmol l^{-1} NH_3 and 18.54 mmol l^{-1} NH_4^+ leads to influxes of both NH_3 (which tends to raise pHi) and NH_4^+ (which tends to lower pHi) that just happen to cancel each other out in terms of their effects on pHi. Because only a small fraction of entering NH_4^+ would dissociate into NH_3 and H^+, whereas almost all entering NH_3 would combine with H^+ to form NH_4^+ (Boron and De Weer, 1976), this scenario would require that the influx of NH_4^+ be far greater than that of NH_3. This is highly unlikely. Nevertheless, to test this hypothesis, we perfused the lumens of gastric glands with a 20 mmol l^{-1} NH_4Cl solution at pH 7.4; that is, one in which [NH_3] was 0.40 mmol l^{-1} and [NH_4^+] was 19.60 mmol l^{-1}. The parallel NH_3–NH_4^+ influx model would predict that, with considerably less NH_3 than in the luminal solution used in the experiment of Fig. 5, this solution would elicit a pHi decrease. Yet we found that this solution had no discernible effect on pHi (not shown).

A second argument is that, rather than being virtually impermeable to NH_3, the apical barriers of gastric gland cells are unusually permeable to NH_3, so much so that virtually all of the NH_3 would leak out of the gland before the luminal solution reached the cells of interest. This is highly unlikely on theoretical grounds, inasmuch as we found that luminal NH_3/NH_4^+ had no effect on pHi, regardless of whether the cells were immediately adjacent to the perfusion pipette or far downstream. Nevertheless, we tested this NH_3-leak hypothesis by perfusing the lumens of glands with the impermeable pH-insensitive dye hydroxypyrenetrisulfonate (HPTS). If NH_3 actually did diffuse out of the lumen, then it should have left behind an increasingly acidic solution. However, when we perfused the lumen with a solution containing 80 mmol l^{-1} NH_3/NH_4^+ at a pH of 7.4, we found that luminal pH was uniformly 7.4 along the entire lumen. We verified that we could easily detect a luminal pH decrease from 7.4 to 7.1. Thus, our control experiments indicate that the apical barriers of parietal and chief cells really were exposed to high concentrations of NH_3 in our experiments.

Effect of CO_2-containing solutions on pHi

Because the above data suggested that the apical barriers of gastric parietal and chief cells have no measurable permeability to NH_3 or NH_4^+, we decided to examine the possibility that these barriers also have an unusually low permeability to CO_2. Fig. 6

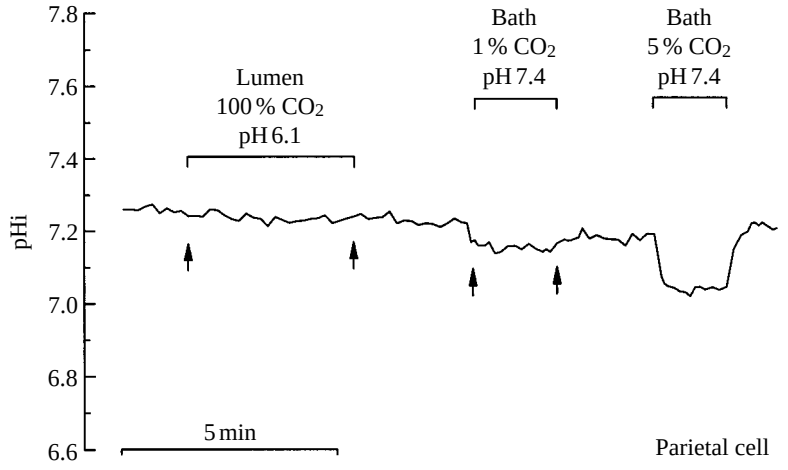

Fig. 6. Effect on parietal cell pH of introducing CO_2/HCO_3^- solutions to either the apical or basolateral surface of the gland. Similar results were obtained on chief cells from the same gland. Reproduced from (Waisbren *et al.* 1994*b*), with the permission of Macmillan Magazines, Ltd.

shows the pHi record for a single parietal cell in an experiment in which we perfused the lumen with a solution containing 22 mmol l^{-1} HCO_3^-, but equilibrated with 100 % CO_2 (pH 6.1). This caused no discernible pHi change in either parietal cells or in chief cells (not shown). In contrast, exposing the basolateral surface of the gland to a solution buffered with 4.4 mmol l^{-1} HCO_3^- and 1 % CO_2 (pH 7.4) produced a small, but reproducible, pHi decrease that averaged approximately 0.08 in a larger series of experiments. An exposure to a 22 mmol l^{-1} HCO_3^-/5 % CO_2 (pH 7.4) solution caused a larger acidification. All basolateral solutions contained DIDS to block HCO_3^- transporters that would have caused pHi to increase after the initial CO_2-induced acidification and would therefore have obscured the CO_2-induced pHi decrease. Similar results were obtained on chief cells.

Fig. 7 shows a false-color pHi image of a gland under control conditions (Fig. 7A) and with the basolateral surface exposed to a DIDS-containing solution equilibrated with 1 % CO_2 (Fig. 7B). Even this low level of basolateral CO_2 caused a noticeable intracellular acidification. Fig. 7C is a control matched in time to Fig. 7D, which shows that 5 % CO_2 in the basolateral solution caused an even greater intracellular acidification than did 1 % CO_2. Finally, a third control pHi image is shown in Fig. 7E; this is matched in time to the image in Fig. 7F, which was taken when the lumen was perfused with a pH 6.1 solution equilibrated with 100 % CO_2. The pHi values are virtually the same in the two images. Except in Fig. 7D, the spiral of relatively acidic cytoplasm that extends down the length of the gland (possibly reflecting the position of the more acidic parietal cells, see above) is more obvious than in Fig. 4.

One unavoidable complication in interpreting any of our experiments is that we cannot distinguish a difference in apical *versus* basolateral permeabilities from a difference in effective surface areas. Estimates of the apical-to-basolateral surface area of parietal cells

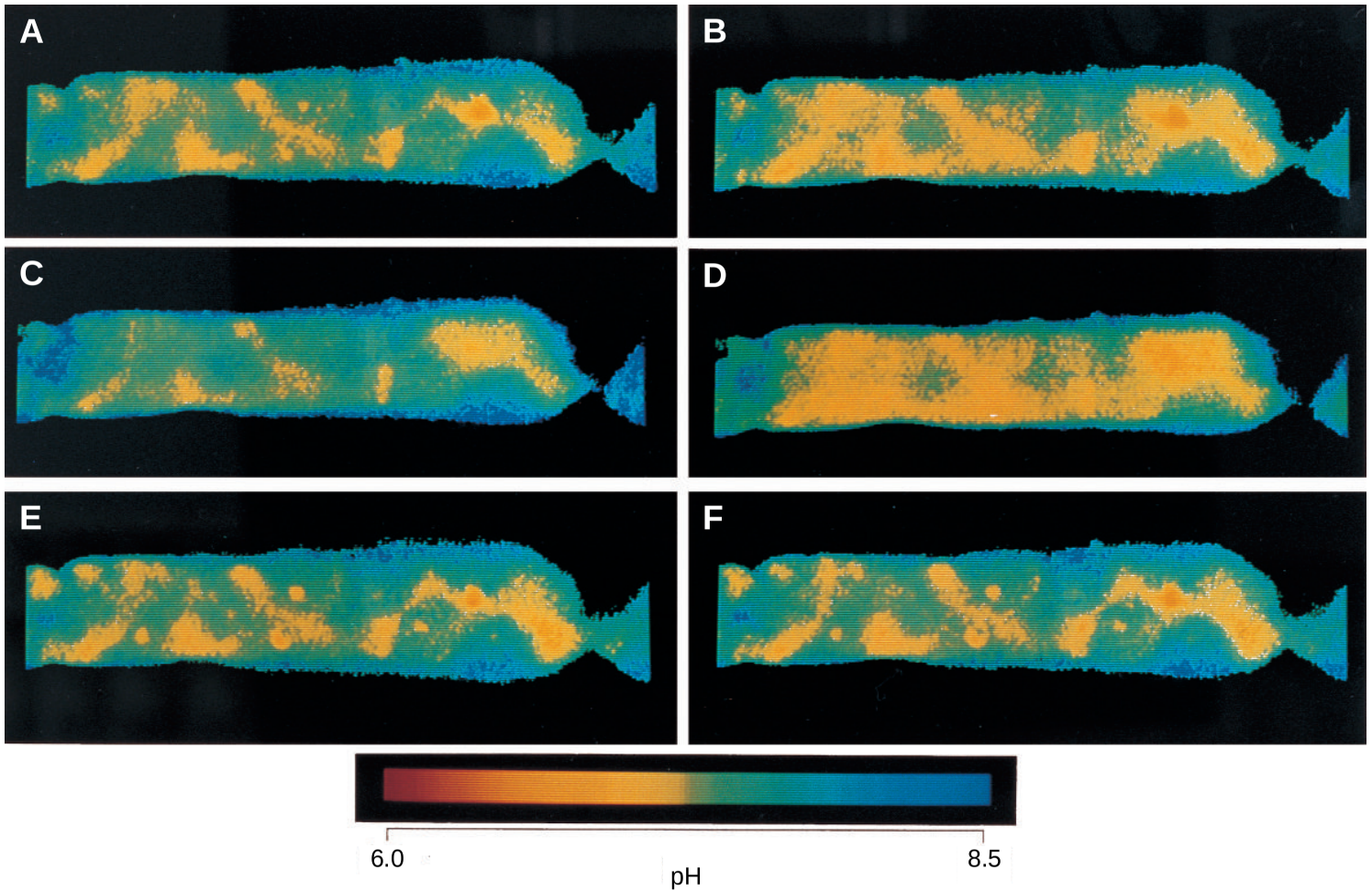

Fig. 7. False-color pHi image of an isolated perfused gland exposed from either the apical or basolateral surface to CO_2/HCO_3^-. The protocol was the same as in Fig. 5. 200 μmol l^{-1} DIDS was present in all basolateral solutions to block HCO_3^- transport. (A) Control conditions. The gland was exposed to a Hepes-buffered pH 7.4 solution from both the apical and basolateral surfaces. (B) Effect of 1 % CO_2 at the basolateral surface. The solution contained 4.4 mmol l^{-1} HCO_3^- and had a pH of 7.4. This image should be compared with the control in A. (C) Control matched in time to image D. (D) Effect of 5% CO_2 at the basolateral surface. The solution contained 22 mmol l^{-1} HCO_3^- and had a pH of 7.4. This image should be compared with the control in C. (E) Control conditions. The gland was exposed to a Hepes-buffered pH 7.4 solution from both the apical and basolateral surfaces. This control image was generated using data obtained near in time to image F. (F) Effect of 100 % CO_2 at the apical surface. The solution contained 22 mmol l^{-1} HCO_3^- and had a pH of 6.1. This image should be compared with the control in E.

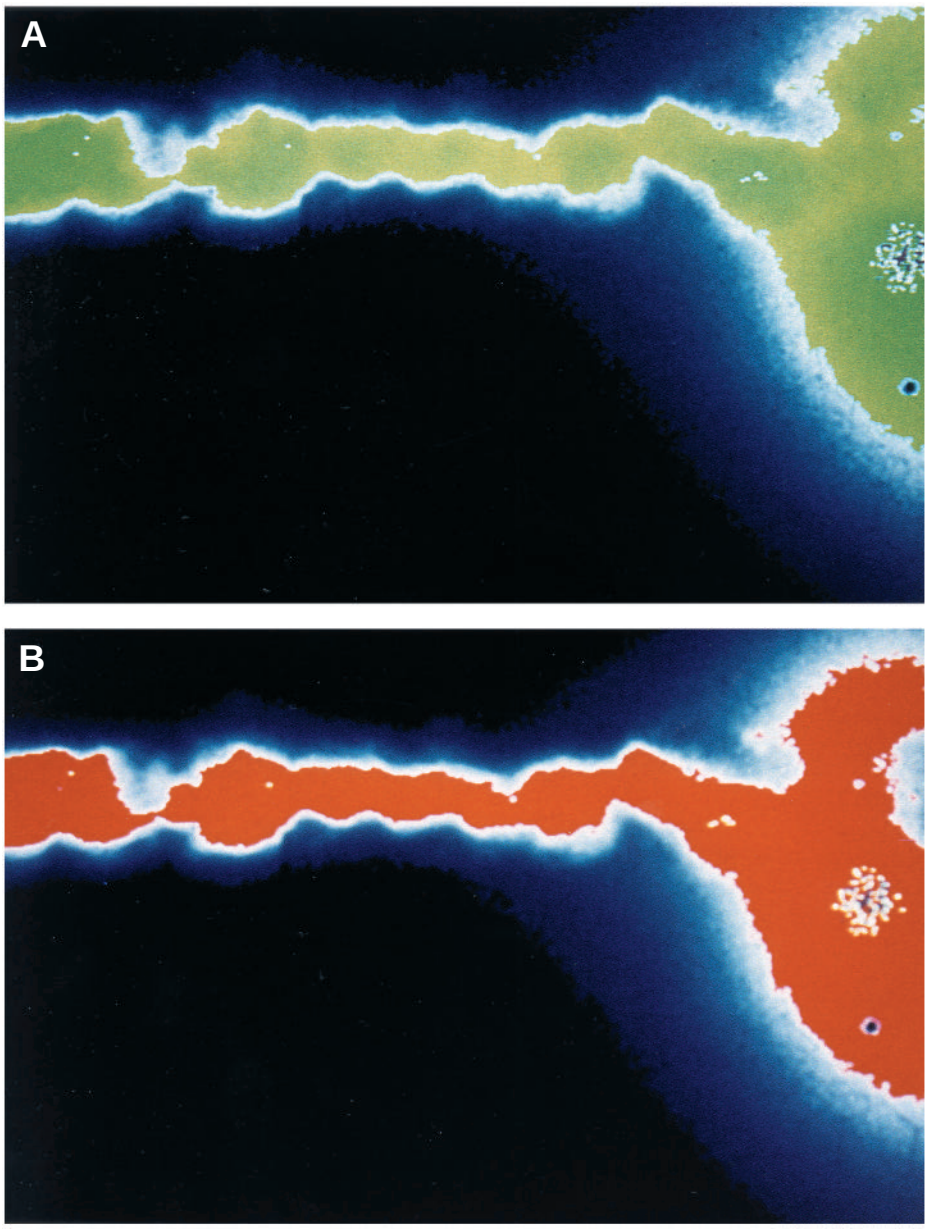

Fig. 8. False-color luminal pH image of an isolated perfused gland perfused with either a pH 7.4 solution buffered with Hepes or a pH 6.1 solution buffered with 22 mmol l^{-1} HCO_3^-/100% CO_2. Green indicates a pH of 7.4 and red a pH of 6.1. The green or red of the lumen is surrounded by a thin envelope of blue that represents a small portion of the cells that surround the lumen. These images give an impression of the tortuosity of the gland lumen, but not of the gland's narrow diameter. (A) Control conditions. The gland was exposed to the Hepes-buffered pH 7.4 solution from both the apical and basolateral surface. The bulbous green region to the right is the out-of-focus lumen of the perfusion pipette. The gland was perfused from right to left. (B) Perfusion with pH 6.1/100% CO_2 solution. Note that the pH of the lumen is uniform from the perfusion pipette lumen to the end of the gland on the left. Had CO_2 leaked out of the gland, the lumen would have become increasingly alkaline as the solution flowed down it.

range from approximately 3:1 in murine parietal cells (Schofield *et al.* 1979) to approximately 1:4 in canine parietal cells (Helander and Hirschowitz, 1972). Thus, our observation that 1 % basolateral CO_2 produces a reproducible acidification, whereas 100 % luminal CO_2 does not, suggests that the difference between the apical and basolateral borders must be greater than what can be accounted for on the basis of surface area. Indeed, a mathematical analysis of our parietal cell data using a model epithelial cell with CO_2 on one side but not the other indicates that the CO_2 permeability $\times$ area product must be at least 1000-fold greater for the basolateral than for the luminal surface.

Of course, the two alternative explanations that could be put forward for our NH_3 data can be postulated for our CO_2 data. For example, it is possible that the apical barriers of parietal and chief cells are permeable to both CO_2 (which would tend to lower pHi) and HCO_3^- (which would tend to raise pHi), and that the opposing effects of these two fluxes merely cancel each other out. However, if this were the case, then the net pHi change should depend on the $[CO_2]/[HCO_3^-]$ ratio. However, we found that neither 100 % CO_2/22 mmol l^{-1} HCO_3^- (Fig. 6) nor 5 % CO_2/22 mmol l^{-1} HCO_3^- (not shown) elicited a change in the pHi of either parietal or chief cells.

A second alternative explanation for our CO_2 data is that the apical barrier of the parietal and chief cells is so permeable to CO_2 that no CO_2 is left in the lumen by the time the luminal perfusate reaches the cells of interest. Once again, this seems to be unlikely on theoretical grounds, inasmuch as we observed no pHi changes in response to 100 % luminal CO_2 regardless of whether the cells were immediately adjacent to the perfusion pipette or far downstream. However, we tested this CO_2-leakage hypothesis by measuring luminal pH while perfusing the lumen with a pH 6.1 solution equilibrated with 100 % CO_2. If CO_2 could indeed leak rapidly out of the lumen, then it would leave behind an alkaline solution. However, we found that switching the luminal perfusate from a pH 7.4 Hepes-buffered solution to the pH 6.1 CO_2 solution caused a large and abrupt decrease in luminal pH that was uniform along the entire lumen. We verified that we could detect an increase in luminal pH from 6.1 to 6.4. The luminal transition from the pH 7.4 Hepes-buffered solution to the pH 6.1 solution buffered with 100 % CO_2 is shown in the false-color images in Fig. 8.

Discussion

Previous work on the apical barrier function of parietal and chief cells

Sachs and his colleagues have compared the passive H^+ permeability of vesicles isolated from the apical membranes of rabbit parietal cells with those isolated from the duodenum and renal cortex (Wilkes *et al.* 1990). The comparison with the duodenum is of interest because the acidic gastric contents are delivered to the duodenum, where they are subsequently neutralized by the secretions from the exocrine pancreas. These authors found that the apparent H^+ permeability of the parietal cell apical vesicles was about one-tenth as great as that of the duodenal vesicles and about one-hundredth as great as that of the renal vesicles. Because even a tiny contamination of low-permeability apical membranes with high-permeability membranes from other parts of the parietal cell would greatly increase the overall permeability to H^+, the data from Sach's group are consistent with a substantial permeability barrier at the apical membrane of parietal cells.

In work on chief cells in culture, Sanders *et al.* (1985) found that a monolayer could maintain a 5 unit pH gradient for over 4 h, provided that the acidic solution was placed at the apical border of the cells. In contrast, if the acidic solution was placed at the basolateral border, the gradient immediately dissipated.

Because the apical membranes in the Sachs and Sanders studies were almost certainly not coated with mucus, these results suggest that the unusual permeability barriers observed in these two studies are at or near the cell membrane.

Permeability versus *surface area*

As noted above, studies of the sort we conducted cannot distinguish between a low apical permeability *per se* and a low apical surface area. Our mathematical analysis of a model epithelial cell exposed to different $[CO_2]$ on the apical and basolateral sides indicates that, if the apical and basolateral membranes have identical areas and CO_2 permeabilities, $[CO_2]_i$ will be the average of the two extracellular $[CO_2]$ values. Thus, if the basolateral membrane is exposed to a CO_2-free solution and the apical membrane is exposed to a solution equilibrated with 100 % CO_2 ($[CO_2] \approx 120$ mmol l^{-1}), $[CO_2]_i$ should be approximately 60 mmol l^{-1}. Our model predicts that as either the apical area or the apical permeability to CO_2 decreases, $[CO_2]_i$ should likewise decrease. Our parietal cell data for the 100 % CO_2 experiments suggest that the area $\times$ permeability product for the basolateral membrane is approximately 1400 times greater than that for the apical membrane. We are unaware of morphometric analyses on chief cells. Moreover, there is some uncertainty as to whether the two studies of parietal cells focused on stimulated or unstimulated glands. However, inasmuch as both studies reported that the ratio of apical-to-basolateral area is near unity, it is likely that the data pertain to the unstimulated parietal cell (i.e. without the introduction of massive amounts of cannulicular membrane). Our CO_2 data were also obtained on unstimulated glands. Thus, if apical and basolateral membrane areas are similar, then our analysis suggests that the effective CO_2 permeability *per se* of the basolateral parietal cell membrane must be at least a few hundred times greater than that of the apical membrane. The same conclusion probably applies to the chief cell.

Do CO_2-impermeable apical membranes confer any physiological benefits on the gastric gland?

Although, from a teleological perspective, it is obvious why the gastric gland should have evolved apical membranes with low permeability to H^+, it is not so clear what the advantage might be of evolving a membrane that has a low CO_2 permeability. Of course, it is possible that the low permeability to NH_3 and CO_2 is an accident. Perhaps whatever renders the apical barriers impervious to H^+ at pH 1.4 renders them poorly permeable to both NH_3 and CO_2. However, it is also possible that there is some advantage to CO_2 impermeability. If the apical borders were CO_2-permeable, then the lumen of the resting (i.e. not secreting HCl) gland would have a $[CO_2]$ somewhere between that of systemic arterial blood (approximately 1.2 mmol l^{-1}) and that of venous blood (approximately 1.4 mmol l^{-1}). If the gland lumen had a pH near 7.4 (i.e. if HCl was not secreted at rest), then the $[HCO_3^-]$ in the lumen of the resting

gland would be somewhere between 20 and 30 mmol l^{-1}. In addition, carbamino formation could increase the total [CO_2] of the gland contents even further. (We note that there are no data addressing the composition of the fluid in the resting gland.) The CO_2-permeability of the gland would have two negative consequences for the sudden stimulation of HCl secretion. First, the secreted HCl would have to neutralize all luminal HCO_3^- before luminal pH could be dropped much lower than 5. Second, this neutralization of luminal HCO_3^- would lead to the formation of CO_2. Even approximately 23 mmol l^{-1} luminal HCO_3^- (with no additional total CO_2 present as HCO_3^- or carbamino compounds) would lead to the formation of enough CO_2 to saturate the aqueous luminal solution. Thus, some of the newly formed CO_2 gas might come out of solution and form microscopic bubbles, occluding the gland lumen (a case of the 'gastric bends'). Unfortunately for the gastric gland cells, this luminal occlusion would be only temporary. The 100 % (i.e. 120 mmol l^{-1}) luminal CO_2 would soon diffuse back into the parietal and chief cells, perhaps with serious or even lethal consequences: the influx of CO_2 into these cells would cause pHi to fall precipitously, at least temporarily.

Titrating HCO_3^- to CO_2 could also be a problem for the intracellular tubulovesicles within quiescent parietal cells. The membranes surrounding these tubulovesicles contain the H^+/K^+ pumps and K^+ and Cl^- channels described in the Introduction. Stimulation of the parietal cell causes the tubulovesicles to fuse with the apical membrane (Forte and Wolosin, 1987), whereas the tubulovesicles presumably reform after the stimulus is removed. If the tubulovesicular membrane were CO_2-permeable, and if the resting pH of the tubulovesicular elements were near neutrality (i.e. if HCl secretion were inactive), then the rapid formation of CO_2 from HCO_3^- before vesicle fusion could lead to vesicle distension or even lysis.

We recognize that, if the apical barriers of parietal and chief cells were CO_2-permeable, the parietal cell could circumvent the negative consequences of sudden CO_2 formation simply by continuously secreting HCl into the gland lumen at a low rate and, thereby, keeping the resting luminal [HCO_3^-] low. Indeed, the stomach does secrete acid between meals although in some subjects the pH of the stomach lumen is not low enough to eliminate HCO_3^-. Moreover, it is not known whether all glands continuously secreted low amounts of acid.

Where is the barrier?

Our work suggests that the basolateral barriers of both parietal and chief cells have normal permeability properties with regard to H^+, NH_3 and CO_2. It is the apical borders that appear to be unique. We are careful to point out that, although it is likely that the permeability barrier lies near the apical membrane, we do not know precisely where. It is possible that the apical membrane has a lipid composition that is so unique that it is impervious to CO_2. However, it is easier for us to conceive of an as yet undescribed barrier (e.g. protein or glycoprotein) on the surface of the apical membrane that renders it impenetrable to H^+, NH_3 and CO_2.

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