Biochimica et Biophysica Acta, 1116 (1992) 43–49

BBAGEN 23643

Bicarbonate and other buffer systems can enhance the rate of H^+ diffusion through mucus in vitro

Mohamed A. Desai and Pankaj M. Vadgama

Department of Medicine (Clinical Biochemistry), University of Manchester, Hope Hospital, Salford (UK)

(Received 9 July 1991)

Key words: Diffusion; Hydrogen ion; Mucus; Permeability; (In vitro study)

The effect of various diffusible buffers on mucus H^+ permeability, and in particular the potency of the HCO_3^-/CO_2 buffer system relative to other selected buffers is reported here. The diffusional resistance of mucus and water was demonstrated to be dependent on buffer concentration, and the contrast between the two types of layer was most pronounced for low D_{H^+} values near neutrality. This concentration dependence was most marked with mucus layers in the buffer systems investigated. Furthermore, the nature and pK_a values of the diffusible buffer systems used in this study had a profound effect on measured D_{H^+} The effect was particularly striking in the case of HCO_3^- buffer with mucus. Possible implications of these in vitro findings in mucosal protection from acid are discussed.

Introduction

Mucus forms a protective gel layer on the surface epithelium of the gastrointestinal tract. Though variable in thickness [1,2], high synthetic rates for its constituent glycoprotein ensure a maintained layer in the face of hostile luminal contents. Accordingly mucus constitutes an integral part of the interface between the luminal contents and the mucosa. There is a general agreement on the importance of mucus as a high viscosity mechanical barrier, but it may have other important protective roles, and its alteration in a number of pathological states (3) may contribute to epithelial damage. Maintenance of an adequate mucus layer is thought by some to be an important means of gastric mucosal protection from luminal acid and peptic digestion [4,5]. A deficiency in mucus may precede and, thereby, play an aetiological role in chronic peptic ulcer [4].

The exact mechanisms responsible for gastric mucosal protection from acid remain to be conclusively determined. Certainly, the overlying mucus layer could be the basis for such mucosal protection, but neither its intrinsic buffering power [7] nor the ability to retard H^+ diffusion [8,9] appear to be adequate to resist ~ 100 mM HCl. Another factor needs to be postulated. Florey [10] considered mucus to be a defence against H^+ by virtue of the HCO_3^- Secreted into it. In this mucus/bicarbonate barrier, mucus is considered to provide an unstirred water layer at the epithelial surface, where local neutralisation of acid occur due to a limited secretion of bicarbonate at relatively low concentrations [11]. Consistent with this hypothesis is the demonstration, with micro pH electrodes, of pH gradients within mucus that is maintained in contact with viable gastric epithelium [12,13]. Bicarbonate concentrations found in the non-parietal component of gastric juice in dogs (8 mmol/l) [14] and in man (25 mmol/l) [15] probably represent the approximate levels of HCO_3^- present within mucus at the epithelial interface. Such concentrations fall well short of the bicarbonate required for HCl neutralisation under ambient conditions generally present in the stomach [16]. A further variable, which may allow the HCO_3^-/mucus barrier to operate effectively is a retarded H^+ diffusion.

Our early studies using mucus-coated glass pH electrodes suggested that H^+ diffusion through mucus was highly pH-dependent [17]. Thus, it appeared that above pH 4 the barrier property of mucus was greater than over conditions usually used to determine mucus H^+ permeability [18–20]. More recently these observations have been confirmed, and quantitated in terms of

Abbreviation: CA, carbonic anhydrase.

Correspondence: M.A. Desai, Department of Medicine (Clinical Biochemistry), University of Manchester, Hope Hospital, Salford M6 8HD, UK.

objective diffusion coefficients [21,22]. Remarkably, we have found that H^+ diffusion through aqueous films is also strongly pH-dependent. Diffusion coefficient/pH profiles show a minimum at pH ~ 7, with D_{B^-} at pH 7 only 10% of D_{H^+} at pH 1 [19], and D_{H^+} for mucus reduced by a further factor to 2%. During these studies we found evidence for the well-known buffer shuttle effect whereby apparent D_{H^+} increases in the presence of diffusible buffer [23,24], particularly when buffer pH is close to pK_a.

Here we report on the effect of different diffusible buffers on mucus H^+ permeability, and in particular the potency of the HCO_3^-/CO_2 buffer system relative to that of other selected buffers. Possible implications of these in vitro findings for mucosal protection from acid will be discussed.

Experimental procedures

The measuring glass pH electrode (Type CETL; Russell, U.K.) was used in conjunction with a saturated calomel reference electrode (Microelectrodes, Londonderry, U.S.A.). Electrode e.m.f. was measured using a pH meter (PCMKI, Newcastle, U.K.) and output recorded at a strip-chart recorder (Linseis, Selb, Germany). A combination pH electrode served as a follower electrode to monitor bulk solution pH during the addition of HCl in the pH jump experiments. All standard reagents were of AnalaR grade and purchased from BDH (Poole, U.K.); carbonic anhydrase (CA) from bovine erythrocytes (EC 4.2.1.1 specific activity ~ 200000 U/mg) was obtained from Fluka (Glossop, U.K.). Native pig gastric mucus from the stomachs of freshly slaughtered abattoir animals, was removed, washed and stored as described previously [22].

Native mucus was applied to the tip of the measuring glass pH electrode which had a pre-mounted 135 μm nylon netting that acted as a spacer. A uniform mucus layer of equivalent depth was then created by stretching a final external 10 μm Cuprophan dialysis membrane, using Cuprophan from a haemodialysis cartridge (Gambro, Lund, Sweden). For measurements through aqueous films, the nylon spacer and the dialysis membrane were used alone. The measuring and follower electrodes were immersed in a mixing chamber containing 175 ml of solution that was stirred rapidly throughout the experiments [22], 1 M NaOH was added to raise the pH of the bulk solution to about 11 prior to HCl addition. When the electrodes had achieved equilibrium, 1 M HCl was added over 1–2 s using an automatic pipette; this produced step changes in pH of the bulk solution of about 1. Solution temperature was maintained at 21 ± 1°C.

Measurement principle

The dynamic response of a pH electrode and its approach to an equilibrium was modelled in terms of diffusion through a stagnant, unstirred layer over the sensor surface [25]. Provided that the diffusion layer, and not the intrinsic electrode response, is rate limiting, the change in the electrode e.m.f. shows the following dynamic time-dependence in its approach to an equilibrium response:

$$E_t = E_{eq} + S \log\left[1 - \left(1 - \frac{(H^+)_0}{(H^+)_{eq}}\right)\frac{4}{\pi} e^{-t/\tau}\right] \quad (1)$$

Here, E_t is the electrode e.m.f. at any given time t, E_{eq} the electrode equilibrium response, S is the slope of the pH calibration graph (mV per decade), $[H^+]_0$ is the H^+ concentration at time zero, $[H^+]_{eq}$ is H^+ concentration at the final equilibrium response and τ is the time constant for the system. The value of τ is governed by both the thickness of the unstirred layer, d, and by the H^+ diffusion coefficient, D, within that layer:

$$\tau = \frac{4d^2}{\pi^2 D} \quad (2)$$

The measurement of τ permits the calculation of D provided that d is known. Alternatively, mucus and liquid films have been created over the glass surface of a pH electrode [21,22], which provided a well-defined boundary layer in a stirred solution and which, furthermore, were of sufficient depth both to define the dynamic response of the pH electrode according to Eqns. 1 and 2 and eliminate the effects of an external Nernst diffusion layer.

Results

The recording of dynamic responses of coated and uncoated pH electrodes, and their treatment to obtain effective diffusion coefficients of HCl (D_{HCl}), have been described and validated previously [21,22]. By applying the same principle, D_{HCl} in various buffer systems at varying concentrations were estimated across a wide pH range.

The results shown in Figs. 1–4 all confirm the general trend, previously observed [21,22] of a strong pH-dependence for apparent D_{HCl} in both aqueous and mucus layers in all the buffer systems examined, notably, bicarbonate (HCO_3^-), phosphate (PO_4^{3-}), glucosamine and a mixture of HCO_3^- and PO_4^{3-}, respectively. The key observation is that, whereas high D_{HCl} is observed at pH extremes, D_{HCl} is low around physiological pH values. Thus, for both aqueous and mucus layers, for example, there is a 5–10-fold reduction in D_{HCl} at ~ pH 7 as compared with that at pH 3.0

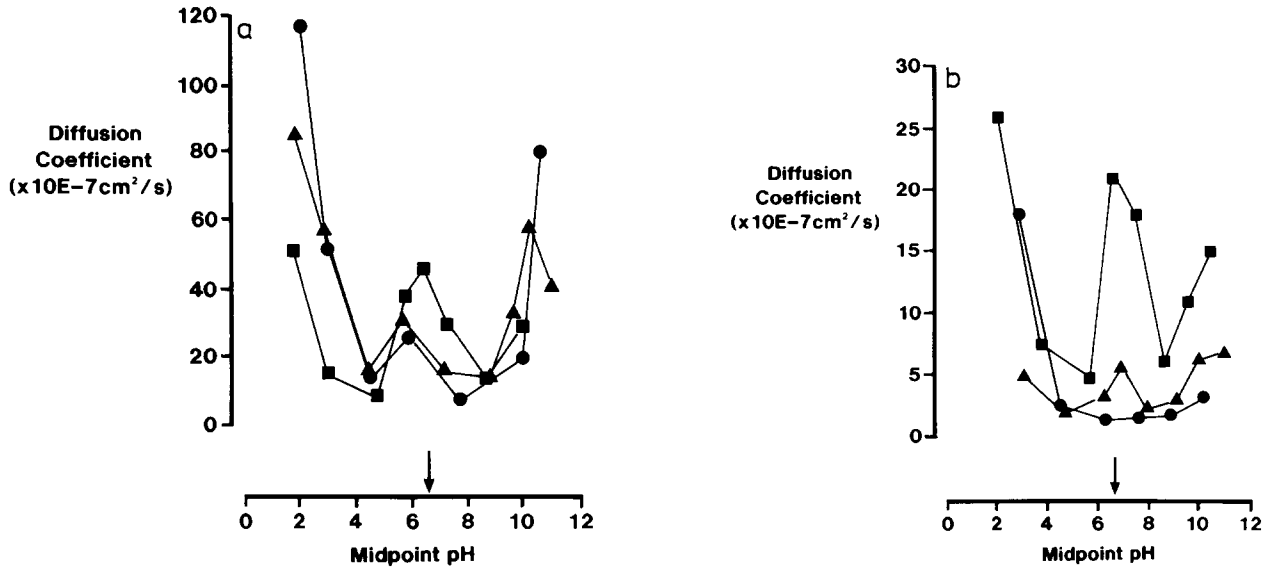

Fig. 1. Effective D_{HCl} calculated from dynamic responses of a spacer and dialysis membrane mounted pH electrode against pH in aqueous layer (a) and mucus layer (b) at various HCO_3^- concentrations; 1 mM (●), 5 mM (▲), and 50 mM (■). Arrow indicates pK_a value of HCO_3^-. pH values are the mid-points of pH jumps.

depending on the buffer system used. In addition, the estimated diffusion coefficients at near neutral pH are approx. 6–15-fold lower through the mucus layer as compared to aqueous layers for lower concentrations of buffer (1 mM) where there would be minimal influence of buffer ions.

The second major observation is that as buffer concentration increases, in most instances there is an apparent direct influence on D_{HCl} around neutral pH values. Thus, as buffer concentration increases, so there is an increase in D_{HCl} (Figs. 1–3). This concentration dependence is most marked on mucus layers (Figs. 1b–3b). Furthermore, the pH at which this maximum impact of buffer is observed is for values in the vicinity of buffer pK_a for each buffer system. Again the effect is most marked on mucus layers as shown in Figs. 1b–4b. The effect for all the buffers is not equivalent and shows the trend HCO_3^- > glucosamine > phosphate. Most dramatic is the high D_{HCl} in bicarbonate at pH ~ 6.5 which is similar to that at pH 3.

The modest effects on D_{HCl} around pK_a (~ pH 7.2) for PO_4^{3-} (Fig. 2), are consistent with the data on mixtures of PO_4^{3-} and HCO_3^- (Fig. 4) and do not suggest any cooperativity or potentiating effect of buffer mixtures. Thus, the overall D_{HCl} values in the buffer mixtures for a combined concentration of 50 mM (Fig. 4) are an order of magnitude lower than D_{HCl} values for HCO_3^- at 50 mM concentration (Fig. 1).

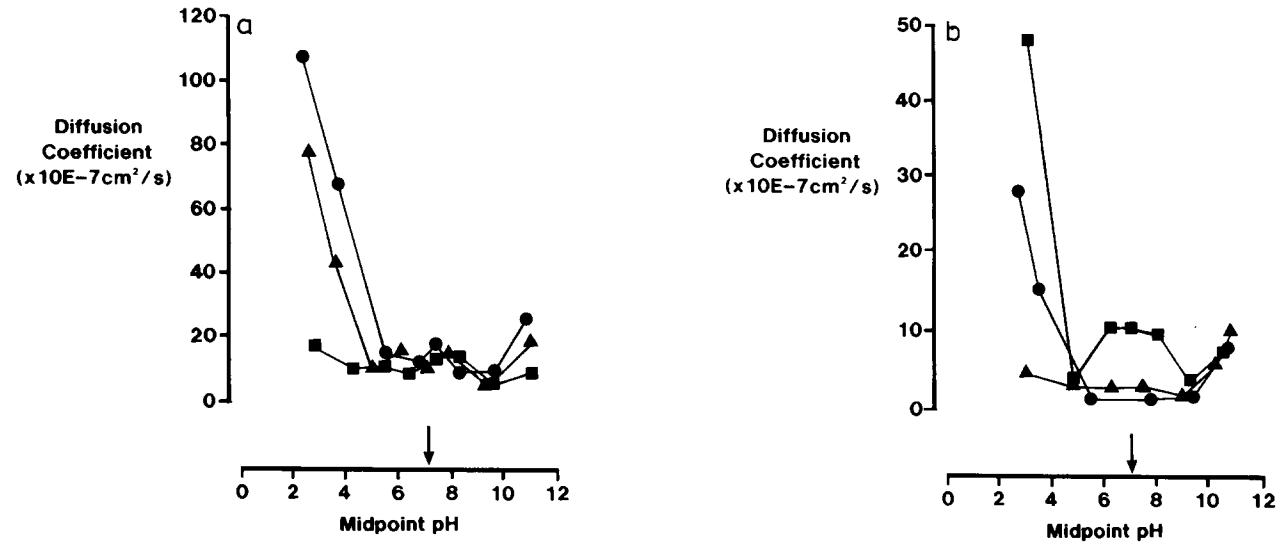

Fig. 2. Effective D_{HCl} against pH in aqueous layer (a) and mucus layer (b) at various PO_4^{3-} concentrations; 1 mM (●), 5 mM (▲) and 50 mM (■). Arrow indicates pK_a value of PO_4^{3-}.

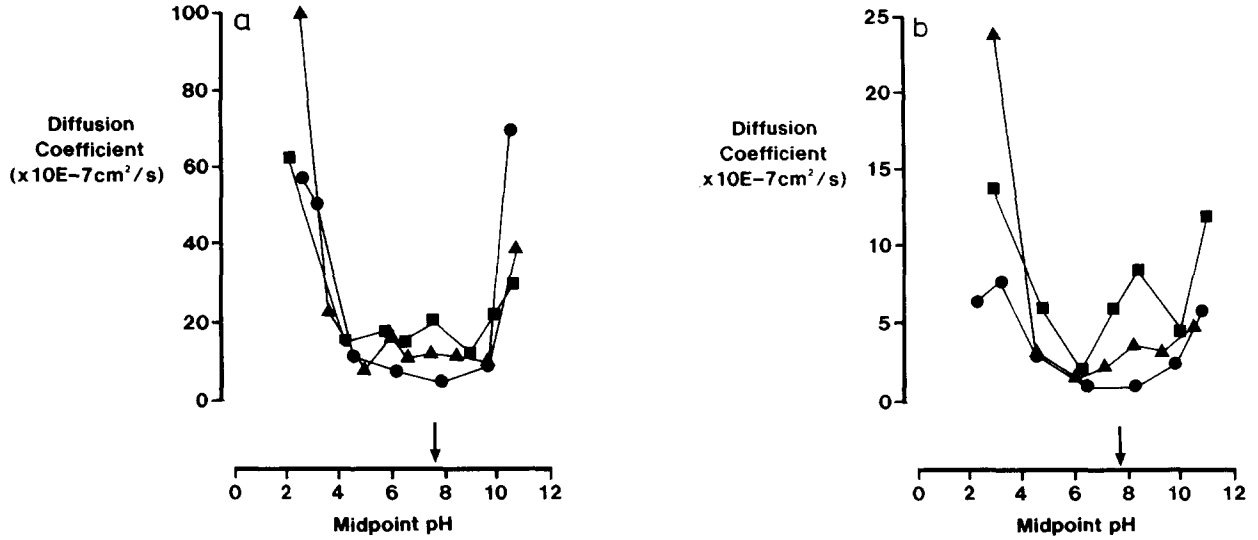

Fig. 3. Effective D_{HCl} against pH in aqueous layer (a) and mucus layer (b) at various glucosamine concentrations; 1 mM (●), 5 mM (▲) and 50 mM (■). Arrow indicates pK_a value of glucosamine.

In order to investigate the effect of carbonic anhydrase (CA) which catalyses both forward and reverse reactions $H_2O + CO_2 \overset{k_1}{\rightleftharpoons} H_2CO_3$, the D_{HCl} experiments were repeated for HCO_3^- buffer in the presence of CA, since the non-enzymic reaction is relatively slow, $k_1 = 0.003$ [26]. H^+ flux at pH $\sim pK_a$ was observed to be independent of HCO_3^- concentrations when CA (1 mg/ml; 200000 U/ml) was present in the bulk solution or in bulk solution and in the liquid film on the electrode surface (Figs. 5 and 6, respectively). Indeed at low pH a reverse trend was seen for D_{HCl} vs. $[HCO_3^-]$ (Fig. 5). Some of the other buffer systems also show such a reverse or at least inconsistent trend with respect to buffer concentrations in the case of both mucus (Fig. 4b) and aqueous (Figs. 1 and 2b) layers. When the HCO_3^- experiments were repeated using CA in both bulk solution and the liquid layer, the results indicate a HCO_3^- independent transport above $[HCO_3^-]$ of 5 mM (Fig. 6).

Discussion

The concept of the mucus-bicarbonate barrier [2,27] has recieved supportive evidence in the form of existence of pH gradients within the mucus layer [27,13] and continues to attract interest [3,4]. Central to finding the importance of this defence mechanism is the diffusional resistance attributable to the mucus gel

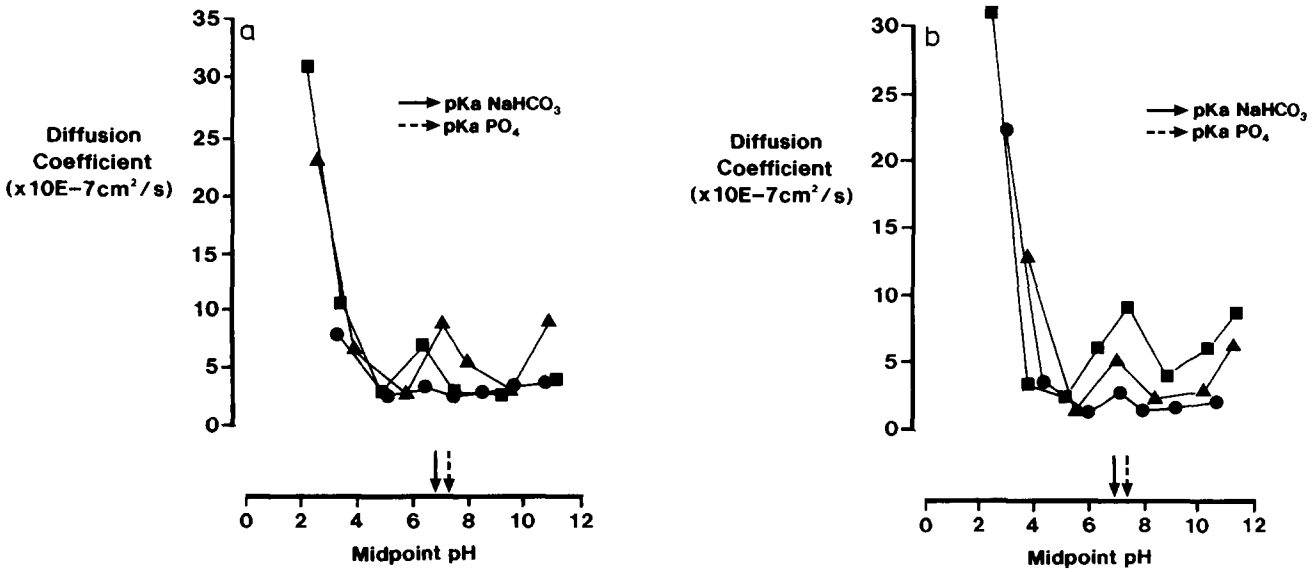

Fig. 4. Effective D_{HCl} against pH in aqueous layer (a) and mucus layer (b) in equimolar mixture of HCO_3^- and PO_4^{3-}; 1 mM (●), 5 mM (▲) and 25 mM (■). Arrows indicate pK_a values of the two buffers.

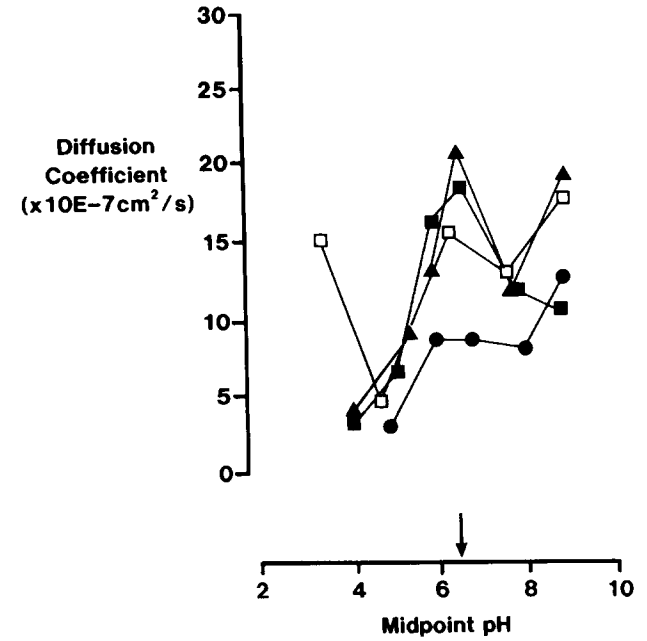

Fig. 5. Effective D_{HCl} against pH in aqueous layer at various HCO_3^- concentrations in the presence of Carbonic anhydrase (1 mg/ml; 200000 U/mg) in the bulk solution only. 1 mM (●), 5 mM (▲), 10 mM (■) and 50 mM (□). Arrow indicates pK_a value of HCO_3^-.

itself; this in turn critically affects the rate at which protons approach the surface epithelium from the lumen, and, therefore, the problem presented to the mucosal surface in relation to proton disposal, through either cellular buffering mechanisms or disposal into the vascular compartment. The major difficulty with the concept of mucus as a diffusion barrier has been that the approximate 4-fold retardation reported hitherto is insufficient to explain the size of pH gradients observed [16], particularly in the face of luminal pH in the 1–2 range. Retardation effects on H^+ diffusion in mucus due to the negative charge of constituent glycoproteins by means of Donnan exclusion mechanism have been found to be minor compared with those of uncharged gels [3], and do not generate a significant barrier. Similarly, diffusional resistance resulting fron the specific ordering of water molecules around the glycoprotein structure [28] or any other colloidal material such as protein, lipid and cell debris [29] is unlikely to have a significant impact on mass transport.

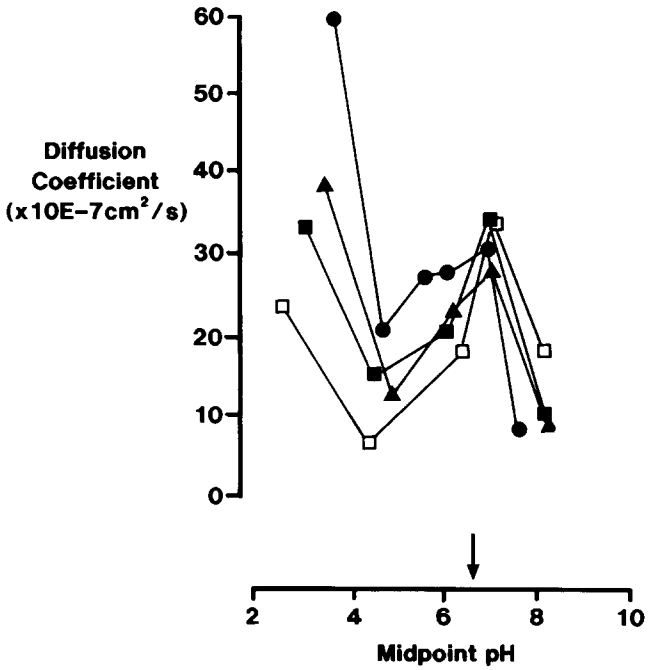

Fig. 6. Effective D_{HCl} against pH in aqueous layer at various HCO_3^- concentrations in the presence of Carbonic anhydrase on the electrode and in the bulk solution. 1 mM (●), 5 mM (▲), 10 mM (■) and 50 mM (□). Arrow indicates pK_a value of HCO_3^-.

The drawback to earlier studies has been, however, the assumption that D_{HCl} is pH independent, and that measurements made at high acid concentrations extrapolate to pH approaching physiological conditions. Certainly, the diffusional resistance of H^+ in mucus observed in this study for low pH (~ 2) and at low buffer concentrations, is consistent with that reported previously by Williams and Turnberg [9], who used a classical two compartment diffusion chamber. However, direct measurement of HCl diffusion coefficients in low strength media have not been reported largely due to the problems of achieving a stable pH under near neutral conditions in a two compartment system without buffering. As a result, all previous studies of D_{HCl} in mucus have been limited to using HCl at or below pH 2. The present single compartment system, however, allows controlled pH in the bulk solution and obviates drift problems by the short < 30 min timescale of the experimental procedure. These are the first such studies, to our knowledge, exploring pH effects on D_{HCl} using this technique.

The results show that the most powerful influence on diffusional resistance is the precise value of pH itself, where a marked drop in H^+ diffusion coefficients occur as neutral pH is approached, irrespective of whether an unstirred liquid or mucus gel barrier is interposed (Figs. 1–4). The effective D_{HCl} values at about neutral pH would appear to have major implications for H^+ diffusion in biological systems generally, and confirm our previous findings [22]. The mechanism for such pH-dependence remains to be elucidated; however, one relevant consideration is the unique mechanism for proton transfer through water, involving the sequential creation/lysis of hydrogen bonds [30].

Essentially, the diffusional resistance of mucus or water in a particular buffer system is concentration dependent, and the contrast between the two types of layers is most for low buffer concentrations near neutrality. Furthermore, the nature and pK_a values of the diffusible buffer systems used in this study have a profound effect on measured D_{HCl}. The effect is par-

ticularly striking in the case of HCO_3^- buffer with mucus as shown in Fig. 1b which shows D_{HCl} to increase with HCO_3^- concentration in the vicinity of HCO_3^- pK_a. However, the effect in PO_4^{3-} (Fig. 2), glucosamine (Fig. 3) and mixture of PO_4^{3-} and HCO_3^- (Fig. 4) though apparent, is less dramatic. This undoubtedly reflects the greater diffusion rates for HCO_3^- and (uncharged) CO_2 through mucus compared to PO_4^{3-} and glucosamine which if less diffusible, would be less able to transport protons through a parallel mechanism of buffer-mediated transport [24,31]. It is possible that PO_4^{3-} with its large negative charge may partition less well into the mucus layer with its negatively charged mucoglycoprotein components as compared with the less negatively charged HCO_3^-. The affinity of pig gastric mucus glycoprotein to counter ions of various charge has been demonstrated previously [32] where ions with high valencies showed the highest avidity for mucus, the opposite effect being operative with ions of opposite charge. Similarly, the possible slow diffusion of glucosamine in this study may be due to specific binding of NH_2 containing glucosamine to mucus. It has already been shown that the diffusion of some antibiotics bearing nitrogen groups are selectively retarded through mucus possibly as a result of specific charge interactions [33]. Such an effect does not explain the reduced buffer influence of PO_4^{3-} and glucosamine in relation to aqueous layers (Figs. 2a and 3a, respectively), although the conclusion that here also HCO_3^-/CO_2 transport is more rapid must still remain a valid one.

Mucus was previously shown in a non-quantitative manner to retard H^+ diffusion above pH 4, and that the barrier property was seriously compromised in the presence of a diffusible buffer [17]. The general effect of a diffusible buffer in solution is well established as a mechanism for augmenting proton transfer by means of a buffer shuttle [24]. This can be represented for bicarbonate by:

$$\text{barrier to protons} \begin{array}{l} H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \text{ low pH} \\ \hline H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \text{high pH} \end{array}$$

This system amounts to an inexhaustible buffer shuttle [31] ferrying protons from a low pH environment to one which is at high pH. All that is required is a pH gradient, and concentration gradients, in opposite directions, for both HCO_3^- and CO_2 reflecting the different pH environments. For HCO_3^-, the source of gradients can be explained on the basis of simple Henderson-Hasselbach equation:

$$pH = pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

whereby, (i) the speed of proton transfer will increase with increasing HCO_3^-/H_2CO_3 buffer concentrations for a given pH gradient, and (ii) the rate of proton transfer is greatest when the pH gradient occurs at or near the pK_a of the buffer i.e. where the maximum gradients of HCO_3^- and H_2CO_3 occur. The overall result is that, as with a buffer in homogeneous solution that minimises pH change, when a pH gradient exists, buffer assisted proton transfer will also act to reduce the size of the pH perturbation [24].

From the above basic principles, the buffer systems under investigation would be expected to operate in a concentration-dependent manner and also to have maximal effect at a pH close to the pK_a of the buffer. This is precisely what has been observed in this study with regards to H^+ flux in various buffer systems, particularly in the presence of mucus. Both phenomena have been observed in a general study of the dynamic responses of pH glass electrodes mounted respectively with immobilised protein [34] and native gastric mucus [17]. This is the first study, however, to explore the bicarbonate buffer shuttle system with mucus.

The additional studies with HCO_3^- in the presence of CA, were carried out to determine the influence of accelerated $H_2O + CO_2 \rightleftharpoons H_2CO_3$ interconversion, both of which are slow reactions. However, CA led to a general reduction of D_{HCl} with increasing $[HCO_3^-]$ at low pH (< 3). D_{HCl} at pK_a was observed to be independent of HCO_3^- concentrations for the aqueous layer. Nevertheless, the flux of H^+ was greatest at the pK_a of HCO_3^-. Interestingly, D_{HCl} at the pK_a was reduced an order of magnitude when CA was present in both the liquid layer and the bulk solution (Fig. 6) as compared with when it was in the bulk solution only (Fig. 5). However, these experiments could not be carried out across the pH spectrum due to potential enzyme inactivation at extreme pH values. The results suggest that in the liquid film, the CA as simply an additional protein component may have retarded H^+ flux. The less efficient transport with CA in bulk solution may be due to the rapid conversion of H_2CO_3 to CO_2, with the latter perhaps requiring additional time to hydrate and liberate H^+ inside the liquid film. CA is present in the surface epithelium at high concentrations [35] and appears to be involved in HCO_3^- secretion since inhibition of this enzyme with acetazolamide reduces alkali secretion [36].

A possible implication of the present findings for the mucus-bicarbonate barrier is that HCO_3^- secretion into mucus by surface epithelial cells may be designed to adjust the pH of the mucus to a range (above 4) where D_{HCl} is reduced, rather than to effect complete neutralisation. At this pH range, the low H^+ diffusion through mucus (reduced $\sim$ 10-fold at low $[HCO_3^-]$) could be sufficient to provide adequate protection of mucosa from acid, and would equally account for the proposed pH gradients in mucus [12,13]. The present

findings therefore provide further support for such a 'modified mucus-bicarbonate barrier' described previously [16,17]. Furthermore, they give indication of why the concentration of HCO_3^- in gastric epithelial secretions is low (approx. 5–10% of maximal acid output [37]). Thus, (i) neutralisation is not necessary, and (ii) a high local HCO_3^- / CO_2 concentrations would have adverse effects by augmenting H^+ flux. Ultimately, the ability of mucosal circulation to cope with an inward diffusion of H^+ from the lumen would be improved if a diffusion barrier were interposed between the mucosa and the lumen, and it is possible that a pH-optimised mucus layer could constitute such a barrier.

These in vitro studies also suggest that a high concentration of buffer within the mucus layer (especially the HCO_3^- / CO_2 buffer system), would greatly accelerate proton flux to the surface epithelium by the operation of a buffer shuttle provided the lumen was acidic. There is also some relevance to the possible role of *Heliobacter pylori* in the aetiology of peptic ulcer. This organism has been repeatedly observed to occur in mucosal surfaces subject to peptic ulceration [38,39]. It is, therefore, of interest that the organism has a high urease activity, and generates ammonia which can certainly neutralise H^+ within mucus [40], but which may also be significant in the associated shuttling of H^+ along the pH gradient contributing to mucosal damage proposed to occur with this organism [41].

References

1 Kerss, S., Allen, A., Garner, A. (1982) Clin. Sci. 63, 187–195.
2 Van Hoogdalem, E.J., de BoeBoer, A.G. and Breimer, D.D. (1989) Pharmacol. Ther. 44, 407–443.
3 Neutra, M.R. and Forstner, J.F. (1987) in Physiology of Gastrointestinal Tract. 2nd Ed. (Johnson, L.R., ed.), pp. 975–1009, Raven Press, New York.
4 Allen, A. and Garner, A. (1980) Gut 21, 249–262.
5 Lee, S.P. and Nicholls, J.F. (1987) Biorheology 24, 565–569.
6 Munster, D.J., Roberton, A.M. and Bagshaw, P.F. (1989) N. Z. Med. J. 102, 607–609.
7 Williams, S.E. and Turnberg, L.A. (1978) Gut 19, A434.
8 Heatley, N.G. (1959) Gastroenterology 37, 313–317.
9 Williams, S.E. and Turnberg, L.A. (1980) Gastroenterology 79, 299–304.
10 Florey, H. (1955) Proc. R. Soc. Lond. (Biol.) 143. 147–160.
11 Allen, A. and Garner, A. (1980) Gut 21, 249–262.
12 Ross, I.N., Bahari, H.M.M. and Turnberg, L.A. (1980) Gastroenterology 81, 713–718.
13 Bahari, H.M.M., Ross, I.N. and Turnberg, L.A. (1982) Gut 23, 513–516.
14 Grossman, M.I. (1959) 21st Int. Congr. Physiol. Sci. Buenos Aires.
15 Makhlouf, G.M., McManus, J.P.A. and Card, W.I. (1966) Gastroenterology 51, 149–171.
16 Engel, E., Peskoff, A., Kaufmann, G.L. and Grossman, M.I. (1984) Am. J. Physiol. 247, G321–G338.
17 Vadgama, P., Covington, A.K. and Alberti, K.G.M.M. (1983) Experientia 39, 573–576.
18 Pfeiffer, C.J. (1981) Am. J. Physiol. 240, G176–182.
19 Lucas, M.L. (1984) Dig. Dis. Sci. 29, 336–345.
20 Turner, N.C., Martin, G.P. and Marriott, C. (1985) J. Pharm. Pharmacol. 37, 776–780.
21 Nicholas, C.V., Desai, M.A., Vadgama, P.M. and Lucas, S. (1991) J. Chem. Soc. Farad. Trans. 87, 293–296.
22 Nicholas, C.V., Desai, M.A. and Vadgama, P. (1991) Analyst 116, 463–467.
23 Vadgama, P. and Alberti, K.G.M.M. (1983) Digestion 27, 203–213.
24 Engasser, J.M., Horvarth, C. (1974) Biochim. Biophys. Acta 358, 178–192.
25 Morf, W.F. and Simon, W. (1978) in Ion-selective Electrodes in Analytical Chemistry. Vol. 1. (Freiser, H., ed.), pp. 211–220, Plenum Press, New York.
26 Ottaway, J.H. and Apps, G.K. (1984) in Biochemistry, 4th Ed., pp. 7–9, Bailliere Tindall, London.
27 Flemstrom, G. and Kivilaakso, E. (1983) Gastroenterology 84, 787–794.
28 Soggett, A. (1980) in Water a Comprehensive Treatise. Vol. 4. (Franks, F., ed.), p. 529, Plenum Press, New York.
29 Slomiany, B.L., Piasek, A., Sarasick, J. and Slomiany, A. (1985) Scand. J. Gastroenterology 20, 1191–6.
30 Hertz, M.G., Braun, B.M., Muller, K.J. and Maurer, R. (1987) J. Chem. Ed. 64, 777.
31 Engasser, J.M. and Wilhem-Despres, A.M. (1980) Chem. Eng. Sci. 35, 669–672.
32 Crowther, R.S. and Marriott, C. (1984) J. Pharm. Pharmacol. 36, 21–26.
33 Niibuchi, J.J., Aramaki, Y. and Tsuchiya, S. (1986) Int. J. Pharmaceut. 30, 181–187.
34 Deem, G.S., Zabusky, N.J. and Sternlicht, H. (1978) J. Membr. Sci. 4, 61–66.
35 Lonnerholm, G. (1977) Acta physiol. Scand. 99, 53–61.
36 Flemstrom, G. (1977) Am. J. Physiol. 233, E1–E12.
37 Rees, W.D.W., Warhurst, G. and Turnberg, L.A. (1981) Gut 22, A882.
38 Blaser, M.J. (1987) Gastroenterology 93, 371–383.
39 Graham, D.Y. and Klein, P.D. (1987) Am. J. Gastroenterology 82, 283–286.
40 Thompson, L., Tasman-Jones, C., Morris, A., Wiggins, P., Lee, S. and Furlong, L. (1989) Scand. J. Gastroenterology 24, 761–767.
41 Yeomans, N.D. (1988) Baillieres Clin. Gastroenterology 2, 573–591.