Mass spectrometric determination of HCO_3^- permeability and carbonic anhydrase activity in intact guinea-pig colon epithelium

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1. A mass spectrometric method originally used in red blood cells was applied to suspensions of isolated colonocytes and intact colonic epithelium to measure the exchange of ^{18}O between HCO_3^-, CO_2 and H_2O to determine intracellular carbonic anhydrase activity (A_i) and membrane bicarbonate permeability (P).

2. In suspensions of isolated guinea-pig colon epithelial cells, colonocytes, we found significantly higher values of A_i and P for cells derived from the proximal colon than for cells from the distal colon. In the case of A_i, this confirms earlier reports.

3. When the ^{18}O exchange process was observed across the mucosal (apical) side of intact colon mucosa, the estimated values of A_i were identical to those obtained for isolated colonocytes, for both the proximal and the distal part of the colon. This is considered to be strong evidence that this method can be applied to a layer of intact epithelium as well as to cell suspensions.

4. The values of P obtained from the apical side of intact colon mucosa were 6 times higher than those estimated from measurements with isolated colonocytes. This indicates that the basolateral membrane of colon epithelium, which participates in the ^{18}O exchange process in isolated colonocytes but not in the ^{18}O exchange process across the apical side of intact mucosa, has a markedly lower bicarbonate permeability than the apical membrane.

5. When the ^{18}O exchange process was observed across the serosal (basolateral) side of intact colon mucosa, the P values, as expected, were low compared with the apical side of intact mucosa. However, rather unexpectedly, the A_i values derived from these measurements were 2–3 times lower than those obtained with isolated colonocytes. It appears possible that the latter finding is an artifact due to the submucosal tissue markedly slowing down CO_2 diffusion from the bathing medium into the epithelial cells, thus causing an apparent fall in A_i.

6. A_i decreased and P increased with increasing temperature, as expected, when studied on the mucosal side of intact colon. This provides additional support for the validity of the method.

In 1940 Mills & Urey described an ingenious method to determine the velocity constants of the hydration of CO_2 and the dehydration of carbonic acid by observing the redistribution of ^{18}O, introduced in the form of ^{18}O-labelled HCO_3^-, into aqueous solution between the pools of HCO_3^-, CO_2 and H_2O. Itada & Forster (1977) applied this technique to the much more complicated situation of a dilute suspension of cells or organelles which contained carbonic anhydrase in their interior. They developed a theory describing the ^{18}O exchange process in this situation which showed that it is possible, by observing the time course of the species $C^{18}O^{16}O$ (mass/charge (m/z) 46 peak height) in the suspension with a mass spectrometer, to determine the intracellular or intra-organelle carbonic anhydrase activity (A_i) and the bicarbonate permeability (P) of the membrane of these cells or organelles (Itada & Forster, 1977; Dodgson, Forster, Storey & Mela, 1980; Dodgson & Forster, 1983). Silverman and associates (e.g. Tu & Silverman, 1975) used the ^{18}O exchange technique in a series of sophisticated studies of the catalytic mechanism of carbonic anhydrase.

It was the aim of the present study to attempt to apply the method of Itada & Forster (1977), developed for isolated cells in suspension, to a layer of intact epithelium. We used

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a situation in which the solution containing the ^{18}O-labelled CO_2 and HCO_3^- is in contact with only one side of the epithelium, either the apical or the basolateral side. Thus, all exchanges including that of $HC^{18}O^{16}O_2^-$ can occur across one side only. Thereby, it should be possible to study differences in bicarbonate permeability across apical and basolateral membranes of polarized epithelia.

The epithelia chosen for this study were the proximal and distal colon of the guinea-pig. Colon can easily be studied from either the apical or basolateral side. This was accomplished by sliding 3 cm long segments of colon onto a Teflon cylinder of suitable diameter, thus effectively excluding the luminal side of the colon segment from any contact with the reaction solution. Using everted colon enabled investigation of the apical side and using non-everted colon exposed the basolateral side to the reaction solution. A major influence in the choice of colon epithelia for this study were reports that bicarbonate permeation across colon epithelium may play an important role in the uptake in the colon of short-chain fatty acids, which are a major nutrient for guinea-pigs and many other herbivorous species (Rechkemmer, Rönnau & von Engelhardt, 1988).

METHODS

Solutions and chemicals

Standard reaction solution contained (mM): 113·6 NaCl, 5·4 KCl, 1·2 $CaCl_2$, 1·2 $MgCl_2$, 0·8 HCl, 2·4 Na_2HPO_4, 0·6 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose. Osmolarity of the solution was adjusted to 300 mosmol l^{-1} using mannitol. pH was adjusted to 7·4.

Buffer solution A, as described by Stieger, Marxer & Hauri (1986) contained: 30 mM NaCl, 5 mM Na_3EDTA, 8 mM Hepes–Tris, 0·5 mM D,L-dithiothreitol (DTT), 40 μg ml^{-1} phenylmethylsulphonylfluoride (PMSF), pH adjusted to 7·6. Buffer solution B, as described by the same authors, contained: 5 mM Na_2SO_4, 1 mM Tris–HCl, 40 μg ml^{-1} PMSF, pH adjusted to 7·6.

PMSF, DTT, ethoxzolamide (EthA) were purchased from Sigma; benzolamide (BA) was from Lederle (Pearl River, NJ, USA); and quaternary ammonium sulphonamide (QAS) was synthesized according to Wistrand (1984). Prontosil–Dextran 5000 (molecular weight, 5000) was prepared as described by Geers, Gros & Gärtner (1985). ^{18}O-labelled bicarbonate, $NaHC^{18}O^{16}O_2$, was prepared as described by Itada & Forster (1977), from $H_2{}^{18}O$ (Euriso-Top, Gif-Sur-Yvette, France). Trypan Blue stain was from Merck. [^{3}H]-inulin was from Amersham–Buchler (Braunschweig, Germany), and unlabelled inulin from Sigma. All other chemicals were of analytical grade and obtained from Merck.

Mass spectrometric determination of A_1 and P in intact cells

Although strictly speaking in mass spectrometry the position of the peak gives the mass/charge (m/z) ratio, since the number of charges on the ionized species is usually 1, m/z is simply its mass. Hence, for ease of presentation in this text m/z will be referred to as m (i.e. $m46$ peak).

Principle. By monitoring the ^{18}O-labelled CO_2, $C^{18}O^{16}O$, and the unlabelled CO_2, $C^{16}O^{16}O$, using mass spectrometry, we used the ^{18}O exchange reaction between ^{18}O-labelled sodium bicarbonate, CO_2 and water to estimate A_1 and P of intact colon cells. Mills & Urey (1940) were the first to use the ^{18}O exchange reaction to determine the rate constants of the hydration of CO_2 (k_u) and of the dehydration of carbonic acid (k_v). They utilized the fact that the ^{18}O exchange reaction approaches isotopic equilibrium nearly two orders of magnitude more slowly than the reaction between CO_2, water, bicarbonate and protons reaches chemical equilibrium. Thus, it was possible to estimate these rate constants by conveniently following the very slow time course of the approach to isotopic equilibrium of ^{18}O between CO_2, bicarbonate and water. Because of the large abundance of water in comparison to the CO_2–bicarbonate system almost all of the ^{18}O, originally introduced into the external solution by adding ^{18}O-labelled sodium bicarbonate, finally ends up in the water pool. This loss of ^{18}O into the water pool causes a decay of ^{18}O-labelled CO_2 (gas with a peak at $m46$), which is slow in the absence of carbonic anhydrase as may be seen, for example, from the initial phases in Figs 2 and 3.

Itada & Forster (1977) showed that the technique of Mills & Urey (1940) could be adapted to measure the intracellular carbonic anhydrase activity in a cell suspension together with the bicarbonate permeability of the cell membranes. They developed a theoretical expression allowing the calculation of these two quantities, and demonstrated the validity of this approach for suspensions of intact red blood cells. They also showed that addition of intact cells containing carbonic anhydrase to the reaction mixture produced a double-exponential disappearance curve for the $m46$ peak (^{18}O-labelled CO_2). Examples of this kind of curve can be seen in Fig. 2*B*, *D* and *E*, Fig. 3*B*, *D* and *E*, and Fig. 4. The fast first phase of disappearance of $C^{18}O^{16}O$ is caused by its diffusion into the cell, where it is rapidly converted into $HC^{18}O^{16}O_2^-$. At the end of this first phase the degree of labelling of intracellular bicarbonate is about equal to that of intra- and extracellular CO_2. Thus, one parameter determining the amplitude and kinetics of phase 1 is A_1 which is defined as the factor by which the CO_2 hydration velocity within the cells is accelerated. During the slow second phase of $C^{18}O^{16}O$ decay one of the processes occurring is the transport of $HC^{18}O^{16}O_2^-$ from the extracellular into the intracellular space. A gradient driving this transport process is present because the chemical reaction between CO_2 and bicarbonate in the extracellular space is uncatalysed and therefore slow. Thus, while extra- and intracellular $C^{18}O^{16}O$ and intracellular $HC^{18}O^{16}O_2^-$ are close to isotopic equilibrium, the degree of labelling of the extracellular bicarbonate is comparatively high at the end of the first phase of $m46$ kinetics. The implication of this is that from the kinetics of phase 2 the P value can be derived. Therefore both A_1 and P can be obtained from measurements such as those shown in Figs 2 and 3. As described by Itada & Forster (1977), two additional parameters that have to be determined independently are required for these calculations: the water fraction of the reaction volume in the chamber occupied by the cells involved in the reaction process, and the surface area of these cells.

All the mass spectrometric results in this paper are presented as semilogarithmic plots, where the relative peak heights are used to calculate $\log(m46/m44 - m46/m44_{end}) = \log m46^*$ which is then plotted *vs.* time. The ratio of $m46$ peak height over $m44$ peak height, i.e. the ratio of labelled over unlabelled CO_2, is used because this eliminates any instability of the mass spectrometer signal for the single gases. $m46/m44_{end}$ represents the state of the final isotopic equilibrium and is obtained by adding excess carbonic anhydrase to the reaction mixture at the end of experiments. As described by Itada & Forster (1977), from plots such as those shown in Figs 2 and 3 we determined the slope of the second phase (n) and the amplitude of the first phase (the step ratio, SR), and used these parameters, along with epithelial cell volume fraction (v), and epithelial surface-to-volume ratio (a) to derive A_1 and P (see Appendix).

Inlet system. We followed the time courses of ^{18}O-labelled and unlabelled CO_2 by means of the special inlet system described by Itada & Forster (1977) which allows continuous sampling of these gases from a small volume of fluid. A water-jacketed glass reaction vessel (1·7–8·5 ml; see Fig. 1) is separated from the ion source of the mass spectrometer (Stable Isotope Detector; Europa Scientific Ltd, Crewe, UK) by a 25 μm thick Teflon membrane which is supported by a sintered glass disc. The Teflon membrane separating the reaction mixture (at atmospheric pressure) from the high vacuum of the mass spectrometer is impermeable to water but highly permeable to gases such as CO_2. The fluid in the reaction vessel is continuously stirred with a magnetic stirrer. Stirring velocity is adjusted to a level which produces a maximum mass spectrometric signal that does not increase with a further increase in stirring speed. Besides minimizing the unstirred layers around cells, stirring is important for reducing the unstirred layer on top of the Teflon membrane. As shown in Fig. 1, the vessel has two (but sometimes three) openings which allow access to the reaction fluid: one through which a glass pH electrode is passed allowing continuous monitoring of the pH in the reaction mixture, the other opening (in some cases two other openings) through which either isolated colonocytes, or a segment of intact colon on a Teflon cylinder, are introduced.

Measurements with intact colon epithelium. The stripped colon (see below) was slid onto a solid Teflon cylinder of suitable diameter (8–10 mm for proximal, 5·4 mm for distal colon). For observation of the apical side of the colon epithelium the segment of colon was everted, and a non-everted segment of colon was used to observe the basolateral side. The colon on the cylinder was immersed and preincubated in standard reaction solution equilibrated with carbogen (95% O_2–5% CO_2).

Solid ^{18}O-labelled sodium bicarbonate (degree of labelling, ~6%) was dissolved in bicarbonate-free, carbogen-gassed standard reaction solution to give a total bicarbonate concentration of 25 mM. The reaction vessel was filled with ^{18}O-labelled bicarbonate standard reaction solution and the pH adjusted to 7·4 by addition of HCl or NaOH. Mass spectrometric recording of the $m44$ and $m46$ peaks was then started. Before the colon was added to the reaction vessel, the slow uncatalysed disappearance of the $m46$ peak was recorded and the slope of this recording used to calculate the velocity of the uncatalysed reaction, which was taken to represent the reaction velocity in the extracellular space which was needed for the calculation of A_i and P.

The cylinder on which the preincubated colon was sitting was attached to a plug and introduced to the reaction vessel (Fig. 1) by replacing the plug used to stopper the opening. The exchange took place as quickly as possible (within 1–2 s) to avoid any significant loss of labelled and unlabelled CO_2 from the reaction vessel. The constancy of the $m44$ signal before and after the exchange of plugs confirmed that such losses were negligible.

Measurements with isolated colonocytes. The pellet from the final stage of preparation of colonocytes, as described below, was weighed, and a known amount of solution added to the pellet to obtain a suspension of the desired cytocrit. The suspension was kept on ice while being gassed with carbogen until used for the experiment. A known volume of the suspension was then added to the reaction mixture in the mass spectrometer vessel to start the measurement.

Preparation of intact colon epithelium and isolated colonocytes

Guinea-pigs. Adult male guinea-pigs (body weight, 550–700 g) were fed a pelleted standard diet (Altromin no. 3122; Altromin, Lage, Germany). Water and food were available *ad libitum*. The animals were maintained on a 12 h light : 12 h dark photocycle. They were killed between 8.00 and 10.00 h by decapitation. Animal experiments were performed in compliance with state and federal legislation.

Intact mucosal epithelium. Proximal and distal colon were removed from the guinea-pigs and flushed with cold carbogen-gassed standard reaction solution to remove luminal contents and then placed into ice-cold carbogen-gassed standard reaction solution. Segments of intact colon (3 cm long) were cut, placed in standard reaction solution and the muscle layer was manually dissected away using forceps. The stripped colon epithelium was then checked for visible damage under a stereomicroscope. The integrity of the stripped colon epithelium was further tested in several cases by staining with Trypan Blue before and after mass spectrometry. The colon segments were stained whilst on the Teflon cylinder, then removed from the cylinder, put into liquid nitrogen and stored at −80°C. From these colon segments 10 μm thick sections were cut on a cryomicrotome, studied and photographed under the microscope. There was no visible Trypan Blue entry into the cells either before or after mass spectrometry.

To estimate A_i and P from the mass spectrometric recordings it is necessary to know the fraction of the total volume of reaction solution which is occupied by the cell water and the surface area of the epithelial cells exposed to the reaction mixture. The mucosal surface area of the epithelium was determined morphometrically from micrographs obtained from colon segments stretched by sliding them onto cylinders of diameters identical to the Teflon cylinders used during mass spectrometry, freezing in liquid

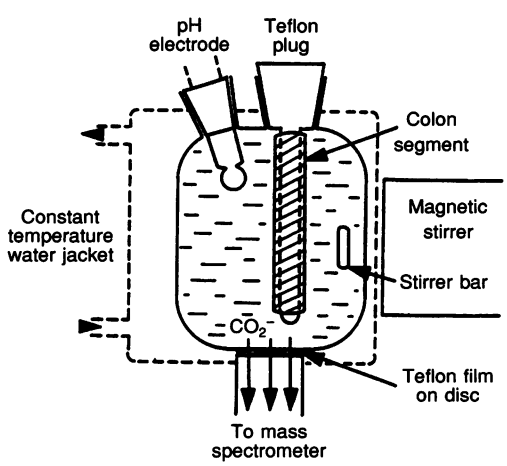

Figure 1. Inlet system to mass spectrometer
Slow diffusion of gases dissolved in the reaction solution across a 25 μm thick Teflon membrane sitting on a sintered glass disc into the high vacuum of the mass spectrometer allows us to record continuously the partial pressures of $C^{16}O_2$ ($m44$) and $C^{18}O^{16}O$ ($m46$). The intact colon segment sits tightly on a Teflon cylinder. When the colon is everted the ^{18}O exchanges with the reaction solution occur across the apical epithelial membranes, when it is non-everted this process is observed across the basolateral membranes.

Table 1. Oxygen partial pressure (P_{O_2}) and critical tissue thicknesses (l_{crit}) in the reaction chamber in the presence of intact proximal colon

	Time = 0	Time = 15 min		Time = 20 min	
	P_{O_2} (mmHg)	P_{O_2} (mmHg)	l_{crit} (μm)	P_{O_2} (mmHg)	l_{crit} (μm)
37 °C	580 (6)	140 (3)	235	120 (3)	218
20 °C	430 (9)	250 (2)	577	180 (7)	490
10 °C	400 (3)	220 (1)	706	290 (2)	810

P_{O_2} values were measured in fluid samples taken from the reaction chamber at the times specified after introduction of intact, stripped proximal guinea-pig colon. The colon was everted and slid onto a Teflon cylinder as described in the text. l_{crit} values were calculated from P_{O_2} using Warburg's equation as explained in the text. These values give the thickness up to which a layer of tissue at a given surface P_{O_2} and with a known specific O_2 consumption can be adequately supplied with O_2 by diffusion. Number of experiments given in parentheses.

nitrogen and sectioning as described. This procedure preserved the degree of stretch that prevailed during mass spectrometry (histological sections show that this stretching ensures that the crypt cells open up, allowing free access by the reaction medium during mass spectrometry). The cryosections were observed under a light microscope at × 100 magnification. The average mucosal surface area was estimated by morphometrically determining the surface area of twenty specimens from representative samples of both proximal and distal colon, and was found to be 1·36 times the surface area of the cylinder onto which the colon had been slid in the case of proximal colon, and 1·50 times in the case of distal colon.

The volume of the epithelial cells participating in the ^{18}O exchange reaction was estimated by multiplying the epithelial surface area by the average height of the epithelial cells of the mucosa in the proximal and distal parts of the guinea-pig colon (Luciano, Reale, Rechkemmer & von Engelhardt, 1984). In a series of measurements we determined by lyophilization the water fraction of epithelial cells to be 85% of the total cell volume, and using this as a factor the water space of the cells was then calculated.

Isolated colonocytes. Colonocytes were prepared by the method of Stieger *et al.* (1986). After removal the colon was rinsed with 0·9% (w/v) NaCl containing 0·5 mM DTT and 40 μg ml^{-1} PMSF and then filled with buffer solution A. The filled colon was incubated in buffer solution A and kept on ice for 20 min. Thereafter, the colon segments were emptied, filled again with buffer solution A and once more incubated in fresh buffer solution A. After 1 h, the solution in the colon was collected and the buffer solution changed. After another hour, the solution filling the colon was again collected. The two solutions collected from the colon interior were combined and centrifuged at 4°C for 7 min at 700 *g*. (Centrikon Rotor A8.24). The pelleted colonocytes were resuspended in buffer solution A by gently shaking and re-centrifuged for 7 min at 700 *g*. The pellet was then resuspended in buffer solution B, as described by Stieger *et al.* (1986), and again centrifuged for 7 min at 700 *g*. After weighing, the pelleted colonocytes were resuspended in a defined volume of bicarbonate-free standard reaction solution.

The fraction of damaged cells in the suspension was determined by Trypan Blue staining directly after preparation or 4 h thereafter. It was observed that the fraction of damaged cells increased over 4 h from 10 to 50%. We therefore confined all experiments to a time period of 2 h after the end of the colonocyte preparation.

The standard method of estimating the surface area and volume of the colonocytes was to measure the average cell diameter under the microscope and use the assumption that the cells can be represented by a sphere. The cytocrit of the colonocyte pellets and the final cell suspension prepared from them was determined as follows. Colonocytes were prepared as described above but, before the last centrifugation, cells were suspended in either unlabelled inulin (4 mM inulin; controls) or ^3H-labelled inulin (4 mM unlabelled with 7×10^{-7} M ^3H-labelled inulin) solutions. Comparison of the radioactivity of the final colonocyte pellets and the cell-free labelled inulin solution allowed us to determine the volume fraction of the pellets that excluded inulin, i.e. the intracellular volume fraction. The control pellets were used to determine the effect of the pellets on counting efficiency; this latter effect was found to be negligible. The fraction of intracellular volume in pellets of both distal and proximal colonocytes was determined to be 30% (average from two preparations).

Oxygen partial pressure in the reaction chamber in the presence of intact colon

Oxygen partial pressure (P_{O_2}) values were determined in the solution in the reaction chamber of the mass spectrometer immediately before and 15 and 20 min after introduction of intact colon into the chamber. P_{O_2} measurements were carried out on small samples from the chamber in an ABL blood gas analyser (Radiometer, Copenhagen) at 37 °C, and the readings were corrected to the actual chamber temperature using the solubilities of O_2 in saline at 37°C and the chamber temperature of interest (Bartels, Bücherl, Hertz, Rodewald & Schwab, 1959). The P_{O_2} values obtained at three chamber temperatures are given in Table 1.

The mean thickness of the proximal colon mucosa of the guinea-pig is 185 μm, and the thickness of the surface epithelial layer is 45 μm; the corresponding values for distal colon mucosa are 230 μm and 35 μm, respectively (Luciano *et al.* 1984). These values can be compared with critical tissue layer thicknesses as they can be estimated from the P_{O_2} values of Table 1 using Warburg's equation:

$$l_{crit} = \frac{2\alpha D \times P_{O_2}}{\dot{V}_{O_2}},$$

where l_{crit} is the critical thickness, α is O_2 solubility, D is the O_2 diffusion coefficient in the tissue, P_{O_2} is the P_{O_2} on the surface of the tissue layer, and $\dot{V}_{O_2}$ is the specific O_2 consumption of the tissue. At the three temperatures 37, 20 and 10°C, respectively: α was

0·0227, 0·0299 and 0·0369 atm^{-1} (Bartels *et al.* 1959); D was $2·2 \times 10^{-5}$, $1·3 \times 10^{-5}$ and $0·7 \times 10^{-5}$ cm^2 s^{-1} (Kawashiro, Nüsse & Scheid, 1975); and $\dot{V}_{O_2}$ was assumed to be 20, 4·6 and 1·8 μl min^{-1} (g wet weight)$^{-1}$. Table 1 gives critical tissue thicknesses for each measured P_{O_2}. It is apparent, from the P_{O_2} values measured after the presence of the epithelium in the mass spectrometer chamber for 15 and 20 min, that at 37 °C (and even more so at lower temperatures) there should be sufficient oxygen to supply the entire mucosa, far above what is needed to supply the surface epithelial cells. Since the mass spectrometric measurements usually lasted for 15 min (for 20 min at most), we conclude that at all temperatures studied the intact colon mucosa was adequately supplied with O_2.

Carbonic anhydrase inhibitors

In the mass spectrometric measurements with isolated colonocytes as well as with intact colon we detected extracellular carbonic anhydrase activity (A_o), which is apparent from the large increase in slope after addition of the cells and the absence of a biphasic time course (Figs 2*A*, 2*C*, 3*A* and 3*C*). In the absence of a biphasic time course it is not possible to estimate A_i and P from the mass spectrometer recording. It was therefore necessary to use extracellular carbonic anhydrase inhibitors to inhibit all extracellular carbonic anhydrase activity without affecting the intracellular enzyme. For this purpose we used Prontosil–Dextran 5000 (Geers, Gros & Gärtner, 1985) at a final concentration of 1×10^{-4} M, the relatively hydrophilic and membrane-impermeable benzolamide (BA) at a concentration of 1×10^{-4} M, or quaternary ammonium sulphonamide (QAS) (Wistrand, 1984) at a concentration of 1×10^{-3} M. At these concentrations all three inhibitors inhibited extracellular carbonic anhydrase activity completely (see Fig. 7), and, as apparent in Figs 2*B*, *D* and *E* and 3*B*, *D* and *E*, this resulted in the appearance of a biphasic time course allowing estimation of A_i and P. In the case of Prontosil–Dextran 5000 the control measurements were performed in solutions containing 10 mM Dextran 5000; this latter concentration was appropriate because in Prontosil–Dextran 5000 only 1/100 of the Dextran molecules are coupled to the sulphonamide Prontosil.

Statistics

Results are expressed as means ± S.E.M. The statistical significance of differences was estimated using the Wilcoxon–Mann–Whitney test.

RESULTS

Time course of $m46$ for intact colon epithelium in the presence and absence of extracellular carbonic anhydrase inhibitors

As seen in Figs 2*A*, 2*C*, 3*A* and 3*C*, addition of intact colon epithelium to the solution containing ^{18}O-labelled CO_2–HCO_3^- resulted in a steep increase in slope of log $m46^*$ *vs.* time; however, there is no evidence for a biphasic time

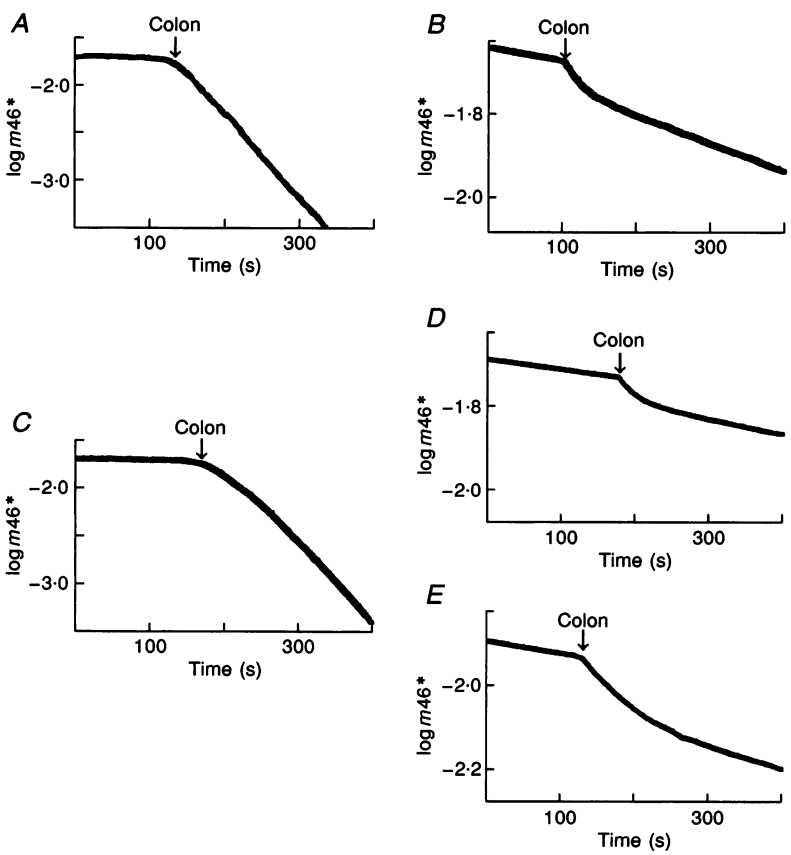

Figure 2. log $m46^*$ *vs.* time for intact everted proximal epithelium in the presence and absence of extracellular carbonic anhydrase inhibitors

$m46^*$ is defined as the peak height ratio $m46/m44$ minus the peak height ratio $m46/m44_{end}$, the latter representing the ratio prevailing after isotopic equilibrium has been reached. *A*, control, 10 mM Dextran alone (no inhibitor); *B*, with 1×10^{-4} M Prontosil–Dextran 5000; *C*, control, standard reaction solution alone (no inhibitor); *D*, with 1×10^{-4} M BA; *E*, with 1×10^{-3} M QAS. Temperature, 20°C.

course of disappearance of ^{18}O-labelled CO_2. Since it is well known that colon epithelial cells exhibit intracellular carbonic anhydrase activity (Lönnerholm, 1977), the most likely explanation for the non-biphasic time course was a rather strong extracellular activity associated with the colon epithelium, which would cause such a pronounced acceleration of extracellular CO_2 hydration that the biphasic time course associated with the intracellular carbonic anhydrase activity of the intact epithelial cells would be obscured. We therefore added Prontosil–Dextran 5000, BA and QAS to the reaction solution to inhibit all extracellular carbonic anhydrase activity (see Fig. 7), but leave all intracellular activity unaffected.

With all three inhibitors a double-exponential decay of ^{18}O-labelled CO_2 was seen similar to that expected in the absence of extracellular carbonic anhydrase for cells exhibiting intracellular carbonic anhydrase activity. The biphasic time courses allowed us to estimate A_i and P for these cells. The results of such measurements in intact distal and proximal colon and in isolated colonocytes are summarized in Tables 2 and 3 and in Figs 5 and 6.

It may be noted that the increase in slope seen upon addition of intact colon in the absence of extracellular carbonic anhydrase inhibitors was much greater in the case of the proximal colon (Fig. 2*A* and *C*) than in the distal colon (Fig. 3*A* and *C*). This indicated that the extracellular carbonic anhydrase activity of the distal colon was much smaller than that associated with the proximal colon.

Comparison of A_i and P derived from intact colon epithelium and from isolated colonocytes

Figure 4 shows mass spectrometric records for isolated colonocytes from proximal (Fig. 4*A*) and distal colon (Fig. 4*B*), which may be compared with records for intact proximal (Fig. 2) and distal (Fig. 3) colon studied from the apical side, and with intact proximal and distal colon studied from the serosal side (Fig. 4*C* and *D*). Table 2 gives a comparison of intracellular activities and bicarbonate permeabilities derived from the intact colon (measurements on the apical side) and from isolated colonocytes; both comparisons are shown for proximal and distal colon epithelium. It is apparent that the calculated A_i values, when derived from measurements on the intact colon epithelium, were virtually identical to those obtained from measurements on isolated colonocytes prepared from the same colon section. The estimated P values, on the other hand, were significantly lower in isolated colonocytes than in the intact colon on its mucosal side, being about 6 times lower in the former compared with the latter. It should be

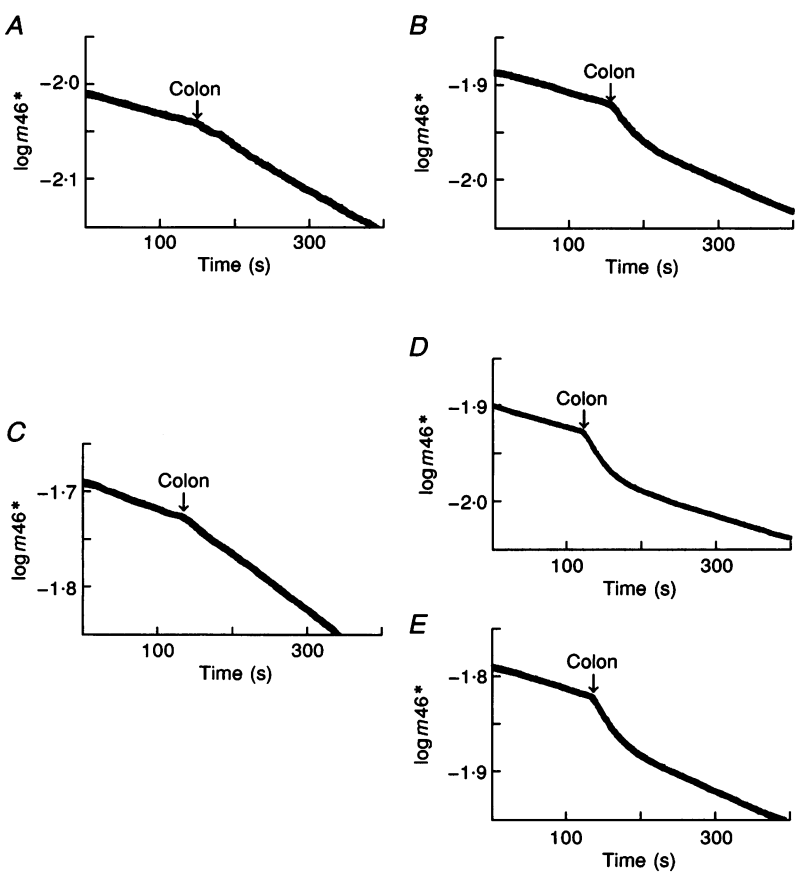

Figure 3. log *m*46* *vs.* time for intact everted distal colon epithelium in the presence and absence of extracellular carbonic anhydrase inhibitors

Conditions in *A*–*E* same as for Fig. 2*A*–*E*. Temperature, 20°C.

Table 2. A_i and P from measurements with intact everted colon and with suspensions of isolated colonocytes

	A_i	P (10^{-4} cm s^{-1})	No. of experiments
Proximal			
Intact colon	460 ± 50	5·8 ± 0·8	20
Colonocytes	470 ± 30	1·0 ± 0·2	13
	n.s.	**	
Distal			
Intact colon	140 ± 10	1·2 ± 0·3	13
Colonocytes	120 ± 20	0·20 ± 0·04	10
	n.s.	**	

A_i is defined as the factor by which the uncatalysed rate of CO_2 hydration is accelerated by carbonic anhydrase inside the cell. The statistical significance of differences between the values for intact colon and those for isolated colonocytes is indicated: n.s., not significant; $^{**}P < 0{\cdot}002$. Temperature, 10 °C.

Table 3. A_i and P from measurements with intact everted and non-everted colon

	A_i	P (10^{-4} cm s^{-1})	No. of experiments
Proximal			
Everted	460 ± 50	5·8 ± 0·8	20
Non-everted	160 ± 10	2·0 ± 0·3	10
	**	*	
Distal			
Everted	140 ± 10	1·2 ± 0·3	13
Non-everted	80 ± 6	1·0 ± 0·1	12
	**	n.s.	

The statistical significance of differences between the values obtained from measurements on the apical (everted) and basolateral (non-everted) side is indicated: n.s., not significant; $^{*}P < 0{\cdot}02$; $^{**}P < 0{\cdot}002$. Temperature, 10 °C.

noted that in the measurements with everted intact colon the properties of only the apical epithelial membrane were determined, while the experiments with isolated colonocytes determined the properties of apical as well as basolateral membranes of the epithelium. So, if the basolateral membrane bicarbonate permeability is lower than the apical permeability, as will indeed be shown below in the case of the proximal colon, then such a difference in permeabilities would be expected. Studying different membrane regions in intact colon and in isolated colonocytes should, however, not affect the intracellular carbonic anhydrase activity determined by this method, and this does indeed seem to be the case.

Comparison of A_i and P obtained from the mucosal and serosal sides of intact proximal and distal colon

The diameters of the Teflon cylinders were chosen so that the colon segments were sitting tightly on the cylinders. Thus, the fluid space between colon and cylinder was minimal, and only the side of the colon in contact with the surrounding reaction medium could participate in the exchange reactions leading to accelerated decay of ^{18}O-labelled CO_2. Therefore, by using either everted or non-everted colon, we could study independently the permeability properties of the apical and the serosal sides of the colon segment. The results of such experiments are shown in Figs 2 and 3 for everted colon, and in Fig. 4*C* and *D* for non-everted colon, and are summarized in Table 3. It is apparent that the mucosal and serosal bicarbonate permeabilities are statistically significantly different for the proximal colon but not for the distal colon. In either case, serosal permeability was lower than mucosal permeability, and for the proximal colon the former was 1/3 of the latter. Similarly, there was a significant difference in calculated intracellular activities when studied from the mucosal and from the serosal side, both for the proximal and the distal colon. Again, the differences amounted to a factor of 2–3. We conclude that for both A_i and P, there are moderate differences between mucosal and serosal measurements. Possible causes for these differences are discussed below.

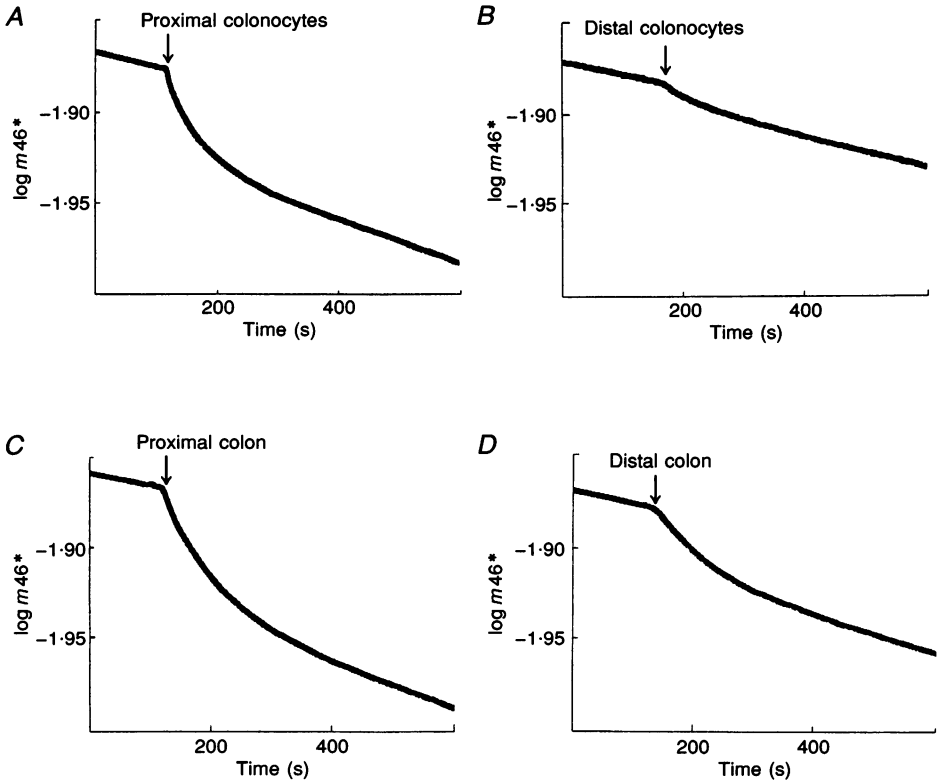

Figure 4. log $m46^*$ *vs.* time for isolated colonocytes and intact non-inverted colon epithelium
A, proximal colonocytes; *B*, distal colonocytes; *C*, non-everted proximal colon; *D*, non-everted distal colon.

Temperature dependence of A_i and P in intact proximal and distal colon

Figure 5 shows, from mass spectrometric measurements at 10, 20 and 37 °C, how the calculated intracellular epithelial carbonic anhydrase activity depended on temperature. It is apparent that the estimated intracellular activity decreases with increasing temperature. This observation was to be expected and was due to the fact that (a) A_i is the factor by which the uncatalysed CO_2 hydration rate is accelerated (Itada & Forster, 1977), and (b) the acceleration of the uncatalysed CO_2 hydration rate with increasing temperature is much more pronounced than the acceleration of the carbonic anhydrase-catalysed CO_2 hydration reaction (Sanyal & Maren, 1981). Therefore A_i, being the ratio of the catalysed plus the uncatalysed rate divided by the uncatalysed rate, was expected to decrease with increasing temperature.

Figure 6 shows the temperature dependence of the bicarbonate permeability, again derived from measurements at 10, 20 and 37 °C with distal and proximal colon. While P did not increase significantly between 10 and 20 °C, there was a highly significant increase in bicarbonate permeabilities

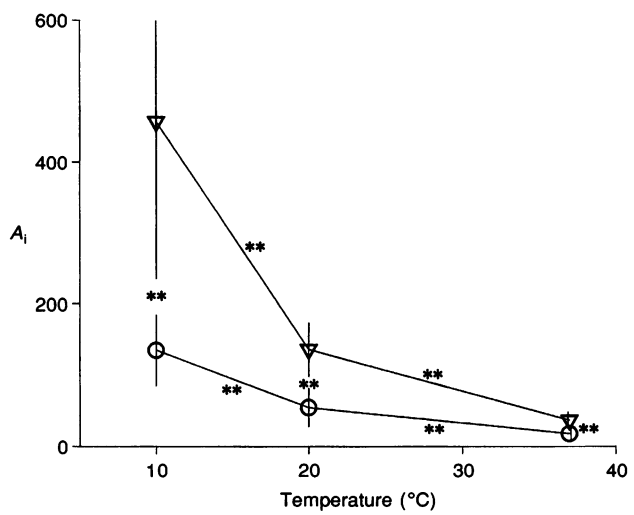

Figure 5. Temperature dependence of A_i in intact, everted proximal (▽) and distal (○) colon
Bars represent S.D. values. Statistical significance of differences between A_i values in proximal *vs.* distal colon and A_i values at different temperatures is indicated: $^{**}P < 0{\cdot}002$.

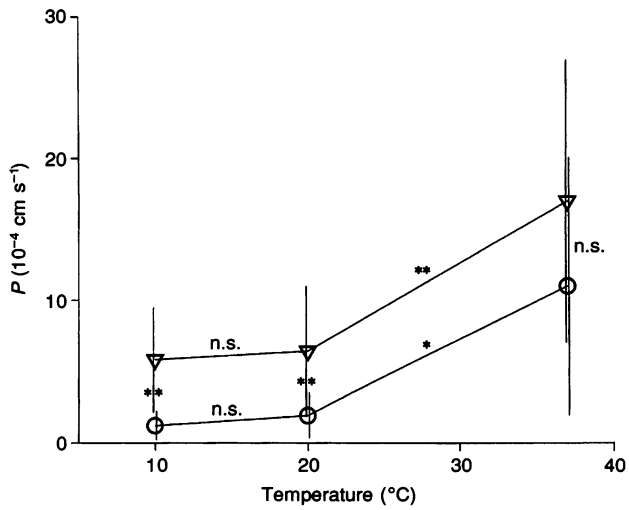

Figure 6. Temperature dependence of P in intact everted proximal (▽) and distal (○) colon
Bars represent S.D. values. Statistical significance of differences between P values in proximal *vs.* distal colon and P values at different temperatures is indicated: $^{**}P < 0{\cdot}002$; $^{*}P < 0{\cdot}01$; n.s., not significant.

when the temperature was raised from 20 to 37°C. We conclude that there was, as expected, a clear increase in HCO_3^- permeability at a higher temperature.

DISCUSSION

Critique of the method

Condition of the cells used in mass spectrometry. In the case of the intact colon segments both the Trypan Blue staining and the P_{O_2} measurements suggested that the epithelial cells remained intact and adequately supplied with oxygen at all stages during the experiments. For isolated colonocytes immediately after the cell preparation we found that 10% of cells were non-vital. This fraction increased with time. Since we confined the use of these cells to 2 h after the end of the preparation procedure, the percentage of disrupted cells occurring in mass spectrometer experiments was estimated to range between 10 and 20%. The non-intact cells were taken into account by the ^{3}H-inulin distribution measurements which give the actual volume fraction of intact cells in the cell pellets and constitute the basis for the calculation of the cytocrit of the cell suspensions, which is the quantity used for the estimation of A_i and P. It may be noted that the estimated values of A_i and P, when plotted as a function of time elapsed after preparation, showed no correlation with this parameter. We conclude that the variation of the volume fraction of intact cells with time after preparation is small enough to produce no significant effect on the calculated parameters.

Carbonic anhydrase inhibitors. Figures 2 and 3 illustrate that the determination of A_i and P requires that rather a large amount of extracellular carbonic anhydrase activity is completely inhibited. Experiments were performed to ensure that the concentrations of the three extracellular inhibitors employed were high enough to achieve full inhibition. An example is given in Fig. 7, which shows a mass spectrometric measurement in which intact proximal colon was added in the presence of half the usual amount of benzolamide (5×10^{-5} M, first arrow). After the fast initial phase of ^{18}O-labelled CO_2 decay was completed, and the trace straightened out indicating the second phase, we added more benzolamide to the reaction chamber to achieve a final

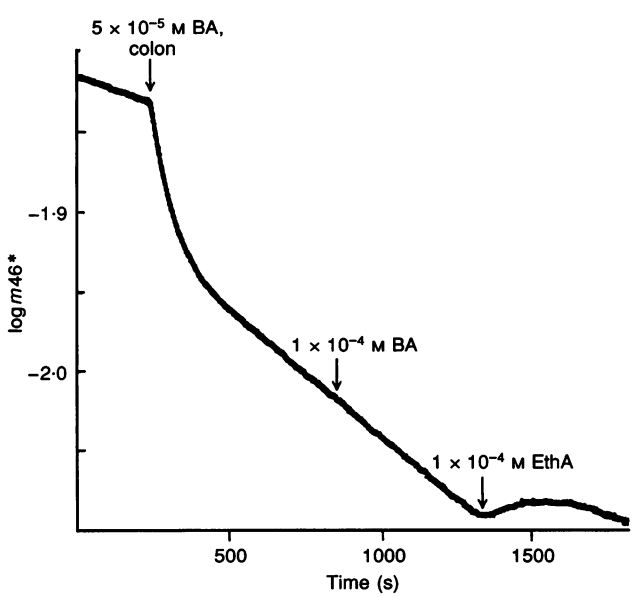

Figure 7. Semilogarithmic representation of an original recording of $m46^*$ *vs.* time for intact everted proximal colon
At time = 0 the reaction solution contained 5×10^{-5} M benzolamide (BA), then the colon segment was introduced (first arrow) producing a typical biphasic time course of $m46$ decay. Raising the BA concentration to 1×10^{-4} M (second arrow) has no effect on $m46$ decay. However, the membrane-permeable carbonic anhydrase inhibitor ethoxzolamide (EthA; 10^{-4} M), produces an immediate transient increase in $m46$, indicating that this inhibitor rapidly blocks intracellular carbonic anhydrase.

benzolamide concentration of 1×10^{-4} M (second arrow). It is apparent that the second addition of benzolamide had no effect on this second phase whatsoever. This would not have been the case had the inhibition of the extracellular carbonic anhydrase effected by 5×10^{-5} M benzolamide not been complete. We concluded therefore that 1×10^{-4} M benzolamide, the concentration that was routinely used, is sufficient for a complete inhibition of extracellular carbonic anhydrase. Analogous experiments leading to the same conclusion were done with the other extracellular carbonic anhydrase inhibitors.

Although the above experiments show clearly that the inhibition of the extracellular carbonic anhydrase was sufficient in our experiments, another question arises. Do any of these extracellular carbonic anhydrase inhibitors permeate the cell membrane and inhibit the intracellular carbonic anhydrase of the epithelial cells during the mass spectrometric experiment? Figure 7 gives a qualitative answer to this question.

The third addition performed in this experiment was injection of 1×10^{-4} M ethoxzolamide (EthA, third arrow), a highly lipophilic and membrane-permeable carbonic anhydrase inhibitor, into the reaction solution. Figure 7 shows that addition of ethoxzolamide leads to an immediate increase in the $m46$ signal, which is an unequivocal indication of a rapid inhibitory effect of this sulphonamide on intracellular carbonic anhydrase activity. Thus, this experiment clearly shows that in the presence of 10^{-4} M benzolamide there is still considerable intracellular carbonic anhydrase activity, which can be inhibited by additionally employing a membrane-permeable carbonic anhydrase inhibitor. Unfortunately, it is not possible at present to evaluate quantitatively the kind of response to ethoxzolamide seen in Fig. 7. Although it cannot be proven from this experiment that the intracellular carbonic anhydrase activity was completely unaffected by benzolamide, the experiment clearly demonstrates that in the presence of benzolamide there was still substantial intracellular carbonic anhydrase activity present. It should be noted in addition that with all three extracellular carbonic anhydrase inhibitors, not only did we obtain very similar biphasic time courses of ^{18}O-labelled CO_2 decay, but also very similar values of A_i and P. In view of the very different structural properties of the three inhibitors, and the very high molecular weight of Prontosil–Dextran 5000, it appears highly unlikely that the identical results for A_i and P obtained with the three inhibitors are due to an identical degree of penetration of these inhibitors into the cells. Rather, it appears likely that none of them penetrates the cell membrane to an appreciable extent and that for this reason they all give identical results.

Cell volume and surface area. The estimated fraction of cell water in relation to the total volume of water in the reaction vessel has a large influence on the calculated value of A_i. The method employed in this paper to determine this quantity was to use the average mucosal surface area obtained morphometrically (surface area of the Teflon cylinder multiplied by either 1·36 or 1·50, see above) and the data taken from Luciano *et al.* (1984) on the height of the epithelial cell layer of the colonic mucosa, where the product of surface area and cell height was taken to represent the epithelial cell volume. Thus, epithelial cell volume was based on experimentally determined quantities for both everted and non-everted intact colon. The surface area, which is a major determinant of the calculated value of P, for everted colon was also directly determined under proper conditions, i.e. we expect that our estimate of the surface area of the colon was reliable when the mucosal (apical) side was exposed to the reaction solution. However, when basolateral membrane permeability was studied in non-everted colon, the same estimate as for the apical surface area was used. This was clearly a substantial underestimate of the true basolateral membrane area. A rough estimate derived from the pictures published by Luciano *et al.* (1984) suggests that the basolateral membrane area may be about 5 times the apical membrane area. Since in the equations used to estimate P (see Appendix) this parameter only occurs as the product of $a \times P$, i.e. surface area-to-volume ratio times permeability, increasing the surface area 5-fold would have resulted in 1/5 the final value for basolateral HCO_3^- permeability. The true basolateral P values, therefore, are expected to be about 5 times lower than those given in Table 3.

It may be noted that in the case of isolated colonocytes, as in the case of everted intact colon, the surface area was determined experimentally. The assumption upon which this determination is based, the spherical shape of the isolated cells, is substantiated by the appearance of the cells under the microscope.

Comparison of A_i and P when derived from measurements in intact colon *vs.* suspensions of colonocytes

Table 2 shows that the values of A_i estimated for intact colon and isolated colonocytes are in excellent agreement, both for proximal and for distal colon. This constitutes strong evidence that the present technique can be applied not only to suspensions of cells but also to layers of intact epithelium with only one side exposed to the reaction medium.

In contrast to the identity of A_i for intact colon and isolated colonocytes, the P values of the mucosal side of intact colon are 6 times higher than those of isolated colonocytes (Table 2). Since the critical parameters, cell volume and membrane area were determined experimentally, as discussed above, both for the everted colon and for isolated epithelial cells, it appears likely that this difference in P is caused by a markedly lower HCO_3^- permeability of the basolateral compared with the apical membrane of these cells. The basolateral membrane in the case of isolated cells takes part fully in the HCO_3^- exchanges, whereas these are restricted to the apical membrane in the case of intact everted colon. It may be noted that part of the apparent high permeability of the apical membrane may be due to the enhancement of the

effective surface area of this membrane by microvilli, which in the present determinations of apical surface area at the light microscopic level are not taken into account. Part of the expected difference in P between apical and basolateral membrane is borne out by the data of Table 3, where P determined from the serosal side is 3 times lower in the proximal and 20% lower in the distal colon compared with the values determined from the mucosal side. As just discussed, the mass spectrometric measurements performed from the basolateral (serosal) side were evaluated using the light microscopic apical (mucosal) surface area, which considerably underestimates the available basolateral membrane area by a factor of approximately 5. Using a more realistic, 5 times greater basolateral surface area, the serosal permeabilities of Table 3 are reduced from 2×10^{-4} to $0{\cdot}4 \times 10^{-4}$ cm s^{-1} (proximal) and from 1×10^{-4} to $0{\cdot}2 \times 10^{-4}$ cm s^{-1} (distal). With these serosal permeabilities, the mucosal permeabilities of Table 3, and an apical to basolateral surface area ratio of 1:5, one predicts an average HCO_3^- permeability of whole colonocytes of $1{\cdot}1 \times 10^{-4}$ for the proximal and of $0{\cdot}37 \times 10^{-4}$ cm s^{-1} for the distal colon. These estimates tally reasonably well with the experimental values of P of isolated colonocytes as seen in Table 2. In conclusion, the difference in HCO_3^- permeabilities of intact everted colon and isolated colonocytes seen in Table 2 does not represent an artifact, but is very probably due to a considerably lower HCO_3^- permeability of the basolateral compared with the apical epithelial membrane.

Comparison of measurements on intact colon from the serosal *vs.* mucosal side

It was shown by Lönnerholm (1977) using the Hanson histochemical technique that in the proximal and distal colon of the guinea-pig, carbonic anhydrase is located only in the epithelial cells of the mucosa, not in the submucosa. The same finding was reported from immunofluorescence stainings by Lönnerholm, Midtvedt, Schenholm & Wistrand (1988). On this basis, therefore, whether we study the intact colon segment from the serosal or the mucosal side, the signal we receive should always be from the epithelial cell layer only, since carbonic anhydrase-free cells have no measurable effect on $m46$ decay.

Why then are the carbonic anhydrase activities in Table 3 lower when measured from the serosal side than when determined from the mucosal side? Observing ^{18}O exchange from the serosal side implies observing this process across a substantial tissue layer, the submucosa, which extends between the epithelial cells and the reaction medium. Since the wet weight of the stripped colon was about 3–10 times the epithelial cell volume estimated as described above (depending on the extent of stripping), the submucosal tissue layer between epithelium and bathing medium was 2–9 times as thick as the epithelium itself. This barrier has to be traversed by all species involved in the ^{18}O exchange process, $C^{18}O^{16}O$, $HC^{18}O^{16}O_2^-$ and $H_2{}^{18}O$. The relative increase in diffusion resistance by this barrier is greatest for the species for which membrane permeability is highest. From studies of the erythrocyte membrane, $P_{CO_2}: P_{H_2O}: P_{HCO_3}$ is expected to be $1 : 10^{-3} : 10^{-4}$ (Forster, 1971; Chow, Crandall & Forster, 1976; Gros & Bartag, 1979). The diffusion coefficients of these species in water, taken as an approximation to those in the submucosa, on the other hand, are not so far apart, the relation being about $D_{CO_2}: D_{H_2O}: D_{HCO_3} \approx 1{\cdot}7 \times 10^{-5} : 2{\cdot}3 \times 10^{-5} : 1{\cdot}2 \times 10^{-5}$ (Moore, 1962; Gros & Moll, 1970; Forster, 1971). Thus, the submucosal tissue layer will cause a much greater relative increase in overall diffusion resistance for CO_2 than for the other species. Since membrane P_{CO_2} is extremely high, the tissue layer leads to an increase of about 1000-fold in apparent membrane diffusion resistance for CO_2. In this situation, the rate-limiting factor in the fast first phase of $m46$ decay may no longer be A_i, but CO_2 diffusion into the epithelial cells. In the theory of Itada & Forster (1977), which is the basis of the calculations of A_i and P, CO_2 equilibration is assumed to be instantaneous. If this no longer holds, then these calculations will yield an apparent A_i that is erroneously low.

This view is consistent with the present finding of 2–3 times lower values of A_i in non-everted compared with everted colon as well as to isolated colonocytes. We suggest therefore that the latter A_i values are correct while those determined from the serosal side of intact colon represent an underestimate.

It may be noted that the same argument suggests that no such problem arises in the determination of P, since the membrane resistance is greater than the submucosal diffusion resistance for HCO_3^-. It appears likely therefore that neither the mucosal permeabilities of Table 2 and 3 nor the serosal permeabilities estimated above are affected significantly by unstirred layer effects.

Comparison of the properties of the proximal *vs.* distal colon

Table 3 allows us to compare intracellular carbonic anhydrase activities and bicarbonate permeabilities for proximal and distal intact colon. It is apparent that, from the mucosal as well as from the serosal side, the estimated values of A_i and P are markedly greater in the proximal than in the distal colon. A_i is about 2–3 times greater in the proximal than in the distal colon, and P is about 2–5 times greater in the proximal compared with the distal colon. With respect to A_i, this result is not surprising. It has been reported previously (Carter & Parsons, 1970*a*, *b*; Lönnerholm, 1977) that the cytosolic carbonic anhydrase activity is greater in the proximal than in the distal colon of the guinea-pig. The difference in proximal and distal bicarbonate permeabilities has not been reported before. However, this finding tallies with the finding of von Engelhardt & Rechkemmer (1992) and von Engelhardt, Burmester, Hansen, Becker & Rechkemmer (1993), who showed that the fraction of short chain fatty acids taken up in the form of undissociated acid is considerably greater in the distal than in the proximal colon. Thus, if we follow the postulate of Mascolo, Rajendran & Binder (1991) and von Engelhardt, Gros,

Burmester, Hansen, Becker & Rechkemmer (1994), that the remainder of short chain fatty acid uptake occurs via a transport mechanism exchanging bicarbonate for short chain fatty acid anions, we would indeed expect that the proximal colon needs a larger transport capacity for bicarbonate than the distal colon.

This idea presumes that the main purpose of the high bicarbonate permeabilities reported here is to allow uptake of short chain fatty acids via short chain fatty acid anion–bicarbonate exchange. This has yet to be investigated.

APPENDIX

This appendix gives the equations that have been used in this paper to calculate carbonic anhydrase activity in the cells (A_i), and membrane bicarbonate permeability (P) from the mass spectrometric records. They are essentially the equations developed by Itada & Forster (1977).

From the ratio of peak heights $m46/m44$, which is proportional to the mass spectrometer signal, the final value of $m46/m44$, $m46/m44_{end}$, as it is reached at isotopic equilibrium, is subtracted, giving $m46^*$. $m46^*$ is plotted semilogarithmically *vs.* time and the amplitude of the fast first phase after addition of cells (step ratio, SR), together with the slope of the second slower phase (n), are determined from this plot.

SR and n are related to A_i and P by the following equations:

$$\mathrm{SR} = 1 + \frac{-m + \left(1 + \frac{A_i v\theta}{1+\theta}\right)\left(0{\cdot}98k_u + \frac{k_v}{1 + K_{HA}/[H^+]_o}\right)}{2{\cdot}3|n| - \left(1 + \frac{A_i v\theta}{1+\theta}\right)\left(0{\cdot}98k_u + \frac{k_v}{1 + K_{HA}/[H^+]_o}\right)},$$

where

$$-m = \tfrac{1}{2}\left(k_u\left(-(A_i v + 1) + \frac{\frac{2}{3}A_i v}{1+\theta}\right) - \frac{k_v}{1 + K_{HA}/[H^+]_o}\left(1 + \frac{A_i v\theta}{1+\theta}\right)\right)$$
$$- \tfrac{1}{2}\left\{\left(k_u\left(-(A_i v + 1) + \frac{\frac{2}{3}A_i v}{1+\theta}\right) - \frac{k_v}{1 + K_{HA}/[H^+]_o}\left(1 + \frac{A_i v\theta}{1+\theta}\right)\right)^2\right.$$
$$- 4k_u\left(-(A_i v + 1) + \frac{\frac{2}{3}A_i v}{1+\theta}\right)\frac{-k_v}{1 + K_{HA}/[H^+]_o}\left(1 + \frac{A_i v\theta}{1+\theta}\right)$$
$$\left. + 4k_u\left(1 + \frac{A_i v\theta}{1+\theta}\right)\frac{\frac{2}{3}k_v}{1 + K_{HA}/[H^+]_o}\left(1 + \frac{A_i v\theta}{1+\theta}\right)\right\}^{1/2},$$

and

$$\theta = \frac{PaK_{HA}}{A_i k_v [H^+]_o},$$

with

$K_{HA} = [H^+][HCO_3^-]/[H_2CO_3]$ = first true dissociation constant of carbonic acid

k_u = (forward) rate constant of CO_2 hydration

k_v = rate constant of H_2CO_3 dehydration

$[H^+]_o$ = extracellular proton concentration (reaction solution)

v = volume fraction of cells

a = surface-to-volume ratio of cells.

It is apparent that these equations provide no explicit result for A_i and P when SR and n are given. We therefore used a trial-and-error procedure to obtain A_i and P from an experimental pair of SR and n.

BARTELS, H., BÜCHERL, E., HERTZ, C. W., RODEWALD, G. & SCHWAB, M. (1959). *Lungenfunktionsprüfungen*, p. 408. Springer, Berlin, Göttingen, Heidelberg.

CARTER, M. J. & PARSONS, D. S. (1970*a*). The carbonic anhydrase of some guinea-pig tissues. *Biochimica et Biophysica Acta* **206**, 190–192.

CARTER, M. J. & PARSONS, D. S. (1970*b*). The purification and properties of carbonic anhydrases from guinea-pig erythrocytes and mucosae of the gastrointestinal tract. *Biochemical Journal* **120**, 797–808.

CHOW, E. I., CRANDALL, E. D. & FORSTER, R. E. (1976). Kinetics of bicarbonate–chloride exchange across the human red blood cell membrane. *Journal of General Physiology* **68**, 633–652.

ENGELHARDT, W. VON, BURMESTER, M., HANSEN, K., BECKER, G. & RECHKEMMER, G. (1993). Effects of amiloride and ouabain on short-chain fatty acid transport in guinea-pig large intestine. *Journal of Physiology* **460**, 455–466.

ENGELHARDT, W. VON, GROS, G., BURMESTER, M., HANSEN, K., BECKER, G. & RECHKEMMER, G. (1994). Functional role of bicarbonate in propionate transport across guinea-pig isolated caecum and proximal colon. *Journal of Physiology* **477**, 365–371.

ENGELHARDT, W. VON & RECHKEMMER, G. (1992). Segmental differences of short-chain fatty acid transport across guinea-pig large intestine. *Experimental Physiology* **77**, 491–499.

FORSTER, R. E. (1971). The transport of water in erythrocytes. In *Current Topics in Membranes and Transport*, vol. 2, ed. BRONNER, F. & KLEINZELLER, A., pp. 41–98. Academic Press, New York, London.

GROS, G. & BARTAG, I. (1979). Permeability of the red blood cell membrane for CO_2 and O_2. *Pflügers Archiv* **382**, R21.

GROS, G. & MOLL, W. (1971). The diffusion of carbon dioxide in erythrocytes and hemoglobin solutions. *Pflügers Archiv* **324**, 249–266.

GEERS, C., GROS, G. & GÄRTNER, A. (1985). Extracellular carbonic anhydrase of skeletal muscle associated with the sarcolemma. *Journal of Applied Physiology* **59**, 548–558.

ITADA, N. & FORSTER, R. E. (1977). Carbonic anhydrase activity in intact red blood cells measured with ^{18}O exchange. *Journal of Biological Chemistry* **252**, 3881–3890.

KAWASHIRO, T., NÜSSE, W. & SCHEID, P. (1975). Determination of diffusivity of oxygen and carbon dioxide in respiring tissue: Results in skeletal muscle. *Pflügers Archiv* **359**, 231–251.

LÖNNERHOLM, G. (1977). Carbonic anhydrase in the intestinal tract of the guinea-pig. *Acta Physiologica Scandinavica* **99**, 53–61.

LUCIANO, L., REALE, E., RECHKEMMER, G. & ENGELHARDT, W. von (1984). Structure of zonulae occludentes and the permeability of the epithelium to short-chain fatty acids in the proximal and the distal colon of guinea-pig. *Journal of Membrane Biology* **82**, 145–156.

MAREN, T. H. (1984). The general physiology of reactions catalysed by carbonic anhydrase and their inhibition by sulfonamides. In *Biology and Chemistry of the Carbonic Anhydrases*, ed. TASHIAN, R. E. & HEWETT-EMMETT, D., pp. 568–579. *Annals of the New York Academy of Sciences* **429**.

MASCOLO, N., RAJENDRAN, V. M. & BINDER, H. J. (1991). Mechanism of short-chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* **101**, 331–338.

MILLS, G. A. & UREY, H. C. (1940). The kinetics of isotopic exchange between carbon dioxide, bicarbonate ion, carbonate ion and water. *Journal of the American Chemical Society* **62**, 1019–1026.

MOORE, W. S. (1962). *Physical Chemistry*, 3rd edn, p. 337. Prentice-Hall International, London.

RECHKEMMER, G., RÖNNAU, K. & ENGELHARDT, W. von (1988). Fermentation of polysaccharides and absorption of short chain fatty acids in the mammalian hindgut. *Comparative Biochemistry and Physiology* **90**, 563–568.

SANYAL, G. U. & MAREN, T. H. (1981). Thermodynamics of carbonic anhydrase catalysis. *Journal of Biological Chemistry* **256**, 608–612.

STIEGER, B., MARXER, A. & HAURI, H.-P. (1986). Isolation of brush-border membranes from rat and rabbit colonocytes: is alkaline phosphatase a marker enzyme? *Journal of Membrane Biology* **91**, 19–31.

WISTRAND, P. J. (1984). Properties of membrane-bound carbonic anhydrase. In *Biology and Chemistry of the Carbonic Anhydrases*. ed. TASHIAN, R. E. & HEWETT-EMMETT, D., pp. 195–206. *Annals of the New York Academy of Sciences* **429**.

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