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Role of Anions and Carbonic Anhydrase in Epithelia [and Discussion]

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Role of anions and carbonic anhydrase in epithelia

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The existence of carbonic anhydrase (carbonate dehydratase, EC 4.2.1.1) in blood was suspected and sought because the rates of spontaneous hydration and dehydration of CO_2 and carbonic acid were slow compared with the rates of exchange of CO_2 with blood. The existence of the enzyme in absorbing and secreting epithelial tissues has, in contrast, often been sought because its presence was required for the operations of theoretical models for the movements of H^+ ions or HCO_3^- into or out of epithelial cells. In addition to the HCl-secreting gastric mucosal epithelium, the enzyme was subsequently found in the rumen, in the kidney, especially those of species that produce acid urine, in salivary gland, the liver and biliary duct system, the mucosa of the small intestine, caecum and colon, the choroid plexuses and ciliary body of mammals, in toad urinary bladder and in the Cl^--secreting cells of fish gill. The presence of carbonic anhydrase in exocrine pancreas does not seem to be well established. The enzyme, of molecular mass about 30 kDa and containing one zinc atom, exists in three related forms: one of high specific activity and two of low specific activity, one of which is found in red skeletal muscle. Although most, but not all, types of erythrocyte contain both varieties, epithelia usually contain only the high-activity enzyme; however, ox rumen contains large quantities of the low-activity variety as do guinea-pig caecal and colonic mucosae. Salt transport in the intestinal tract is associated with movements of HCO_3^- and of H^+ ions, yet although carbon dioxide stimulates solute and fluid transport in the gall bladder and jejunum, and inhibitors of carbonic anhydrase reduce fluid and ion transport across many epithelia, the role of the enzyme in epithelial transport is not clearly understood. Knowledge of the rates of hydration and dehydration of CO_2/HCO_3^- in the fraction of the tissue water responsible for the H^+–HCO_3^- movements in many secretory epithelia is currently lacking.

Introduction: carbon dioxide and the formation of carbonic acid

Fluid moving across epithelia either as absorption from the lumen of an organ into the blood or as secretion from the blood into a cavity or a duct system is primarily always approximately isotonic with the fluid from which the absorbate or secretion originates, although the fluid emerging from the end of a duct or tubule may be of a very different tonicity. During such fluid movements, changes in acid–base balance are found to occur in the fluids on either side of the epithelium; these changes, although by no means as dramatic as those observed during acid secretion by the stomach, seem to be characteristic of ion transport across many epithelia and to involve apparent movements of HCO_3^- and of protons. As will be seen below there is good evidence in the small intestine that the metabolism of CO_2 is closely related to the movement of salt.

By the 1890s it was well appreciated that the CO_2 entering the blood from the tissues is conveyed to the lungs in the form of HCO_3^- ions and that in the lungs the HCO_3^- is dehydrated and the CO_2 released into the alveoli (see Pembrey 1898). It was also known that the reaction

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \qquad (1)$$

occurred at an appreciable rate in the absence of any catalyst. However, doubts were raised as to whether the rates of spontaneous hydration and dehydration of CO_2 were adequate to account for the rates of exchange of respiratory CO_2 observed in mammals. The kinetics of the spontaneous reaction are now well understood and the velocity constants of the various reactions have been measured at a number of temperatures (Roughton 1964; Edsall & Wyman 1958; Edsall 1969). Thus we now know that with the velocity constant (k_{CO_2}) = 0.131 s^{-1} (37 °C) (Roughton 1964), the forward reaction would be 50% complete in 5.3 s, 90% complete in 17.6 s, 99% complete in 35 s, and 99.9% complete in 70 s, although the erythrocytes dwell in mammalian lungs for not more than 1 s. This sort of argument formed the basis of the search for a catalyst to speed up the reactions. Carbonic anhydrase (EC 4.2.1.1; also known as carbonate dehydratase) was identified in and isolated from erythrocytes in the early 1930s by Roughton and his colleagues in Cambridge (Brinkman *et al.* 1932; Meldrum & Roughton 1933; Roughton 1943). It is to be noted that, for physiological purposes, the spontaneous reaction has to be speeded up to 50–100 times, yet there is a vast excess of enzyme in mammalian red cells, an excess of perhaps 500 times.

The values of the velocity constant for the forward reaction (equation (1)) (k_{CO_2}) are very temperature-sensitive. Even allowing for the increased solubility of CO_2 at lower temperatures the spontaneous rate for formation of H^+ and HCO_3^- ions at constant p_{CO_2} is still very temperature-sensitive (table 1), a fact that may be relevant to the reported presence of carbonic anhydrase in gills and the anuran bladder (see below).

TABLE 1. EFFECTS OF TEMPERATURE ON THE SPONTANEOUS RATE OF HYDRATION OF CO_2

(Also given are the rates of hydration at constant p_{CO_2} relative to the spontaneous rate at 0 °C. Values of k_{CO_2} are from Roughton (1964).)

	0 °C	15 °C	25 °C	38 °C
rate constant of forward reaction, k_{CO_2}/s^{-1}	0.002	0.013	0.037	0.130
solubility of CO_2 at 760 mm p_{CO_2} (volume ratio)	1.71	1.02	0.76	0.55
relative rate of forward reaction at constant p_{CO_2}	1.00	3.89	8.22	20.9

CARBONIC ANHYDRASE AND EPITHELIAL TRANSPORT OF HCO_3^-: THEORY

Whereas the search for the enzymic catalysis of the exchange of CO_2 with blood was based on kinetic data, the existence of the enzyme in tissues that acidify was sought because it played a role in models for the movement of H^+ ions and of HCO_3^- ions into and out of tissues.

A few years after the discovery of the presence in erythrocytes of a catalyst for the hydration and dehydration reactions, the enzyme was sought in an epithelium, the gastric mucosa. Berend (1937) argued that with HCl being secreted into the gastric lumen, there should be a corresponding exchange of Cl^- for HCO_3^- between the blood and epithelium. Berend did not search for this exchange, which has been observed (Davenport 1946; Davies 1951), but instead looked in the mucosa for carbonic anhydrase, the catalyst that would generate the necessary protons and HCO_3^- from CO_2 and H_2O. He found the enzyme in the gastric mucosae of dog, cat and rabbit (Berend 1937, 1938), but, although he was careful to perfuse the vessels with Ringer solution to remove erythrocytes, he attributed the quantities of enzyme found to the residual red cells and not to its presence within the epithelial cells. Davenport & Fisher (1938) and Davenport (1939) then showed clearly that the enzyme was indeed present in the gastric

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mucosa including the oxyntic cells; carbonic anhydrase was subsequently shown to be present in the renal tubule (Davenport & Wilhelmi 1941), where it was considered to be an enzyme associated with acidification of the urine.

The models that required the presence of carbonic anhydrase were based on reaction (1) driven by carbonic anhydrase, followed by the instantaneous reaction

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-, \qquad (2)$$

the ions separating to move across the plasma membranes bordering the opposite poles of the cell. Thus the protons might move out across one pole accompanied by a Cl^- ion or in exchange for a cation; across the other pole HCO_3^- would move out either in exchange for an anion, e.g. Cl^-, or accompanied by the cation for which the secreted proton was exchanged across

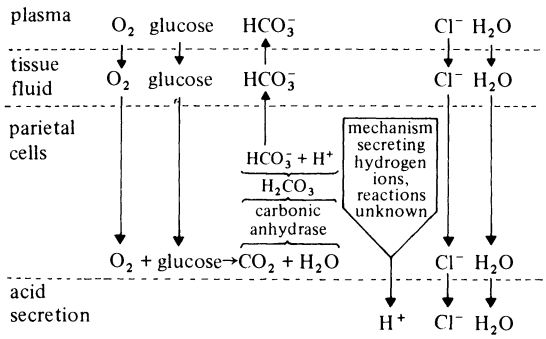

FIGURE 1. Model proposed by Davenport for secretion of acid by parietal cells. Note that the necessary CO_2 was entirely derived from the oxidation of glucose. (Redrawn from Davenport (1943).)

the opposite membrane. Three consequences followed from such models: (*a*) the rate of secretion of protons or of HCO_3^- ought to be faster than the uncatalysed rate of reaction (1); (*b*) carbonic anhydrase ought to be present in the cells involved in the secreting process; (*c*) the addition of inhibitors of carbonic anhydrase at concentrations sufficient to inhibit the enzyme by, say, 99 % should reduce the secretion rate to values in accord with the rate constants of the uncatalysed reaction (1). A further assumption that seems to be implicit in such models is that the diffusion of carbon dioxide from the blood is not rate-limiting to the reactions in cases where the rate of endogenous CO_2 production is insufficient to provide the requirements for CO_2 (figure 1).

Davenport (1946) calculated that the rate of acid secretion by rat oxyntic cells is of the same order of magnitude as that of the uncatalysed reaction at 37 °C, so that if the enzyme is a necessary component of the secretory system, large quantities would seem not to be required.

In table 2 are shown the rates of H^+ or HCO_3^- secretion found in some epithelia together with the fraction of the tissue mass that would be required for the uncatalysed reaction to provide the secretion at the observed rate. It would seem at first sight from these calculations that only for the parietal cells is the spontaneous rate of hydration scarcely adequate to sustain the observed rate and that the CO_2 requirements do not seem to be very different from the basal CO_2 production. However, in considering these data it is a fact that reaction (1) is catalysed by anions of weak acids and bases containing heterocyclic nitrogen, so that even in the absence of carbonic anhydrase, the spontaneous rate of the forward reaction in tissues may be faster than in aqueous solution. Other serious difficulties of interpretation arise because it is

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TABLE 2. RATES OF H^+ OR HCO_3^- SECRETION BY VARIOUS EPITHELIA

	wet mass	rate of secretion			
		μmol h^{-1}	μmol h^{-1} g^{-1} wet mass	ϕ	reference
	g	H^+ ions			
rat stomach					
mucosa	0.5	50	100	0.19	1
parietal cells	0.065		770	1.45	1
		HCO_3^-			
rabbit salivary gland	0.75	66	88	0.17	2
rat pancreas	1.0	12	12	0.02	3
cat pancreas	5	500	100	0.19	4
rat colon	—	152*	30†	0.06	5
guinea-pig ileum	—	1200*	240†	0.45	6
endogenous CO_2 production: intestine	—	—	100‡		
pulmonary CO_2 excretion (human basal)			750§		
uncatalysed rates (38 °C)					
$CO_2 + H_2O \rightarrow H_2CO_3$			470‖		
$CO_2 + OH^- \rightarrow HCO_3^-$			61¶		

References: 1, Davenport (1946); 2, Case *et al.* (1980); 3, Gelinas *et al.* (1982); 4, Case *et al.* (1979); 5, Parsons & Powis (1971); 6, Powell *et al.* (1968).

* Units: per gram dry mass.
† Recalculated on basis that wet mass/dry mass = 5:1 (Parsons & Powis 1971).
‡ Q_{O_2}, small intestine, taken as 10 μl mg^{-1} dry mass h^{-1}, wet mass/dry mass = 4.5:1 and r.q. = 1.
§ Calculated on basis of 220 ml CO_2 min^{-1} basal CO_2 production; wet mass of lung: 800 g.
‖,¶ Units: micromoles per millilitre of water per hour; rates calculated on the basis that $[CO_2]$ = 1 mM.
(‖) k_{CO_2} = 0.131 s^{-1} and (¶) pH = 7.4 and k_{OH^-} = 20 kmol s^{-1} (Roughton 1964). ϕ, fraction of wet mass as tissue water that would supply, by uncatalysed reaction at 37 °C, 530 (= 470 + 60) μmol HCO_3^- per gram wet mass of tissue per hour.

difficult to measure the exact rate of secretion at the cell surface and to assign the fraction of the tissue that is responsible for the secretion. Thus in the stomach some H^+ ions may diffuse back to be neutralized; in the intestine the HCO_3^- that is secreted may react with protons secreted by a separate process (see below). In glands with ducts, the primary secretion can be considerably modified during passage along the ducts. In spite of all these reservations it seems that the true rates of secretion of HCO_3^- and of protons may not be strikingly greater than the uncatalysed rate of hydration of CO_2.

CARBONIC ANHYDRASE: STRUCTURE AND PROPERTIES

After the discovery of the enzyme in erythrocytes (Brinkman *et al.* 1932) the protein was partly purified, found to have a molecular mass of about 30 kDa and to contain one atom of zinc per molecule. Later, the existence of multiple forms of the enzyme was recognized, and, on the basis of differences in the electrophoretic mobilities, forms called A, B and C were identified in human erythrocytes; the A and B enzymes have relatively low specific activity and a common amino acid sequence whereas the C type has a high specific activity and a different amino acid sequence. The nomenclature of the various isoenzymes can be somewhat confusing. Here, in accordance with current usage, the low-activity type (B) will be referred to as CAI, the high-activity type (C) as CAII; more recently a third type (CAIII), also of low activity and which has properties of an acid phosphatase, has been found in mammalian and avian red skeletal muscle; on the basis of the amino acid sequences this type is thought

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TABLE 3. SOME PROPERTIES OF THE THREE MAJOR VARIETIES OF CARBONIC ANHYDRASE

((i), Kinetic data for bovine enzymes recalculated from Tashian *et al.* (1980); (ii), guinea-pig enzymes assayed at pH 7.4 and 0 °C (data of Carter & Parsons 1972); (iii), data for human enzymes and rabbit skeletal muscle at pH 7 and 25 °C (Khalifah 1971).)

	CA I	CA II	CA III
relative specific activity			
(*a*) CO_2 hydratase (i)	3.2	100	1.1
(ii)	2.5	100	—
(*b*) p-nitrophenylacetate hydrolase ('esterase') (i)	40	100	0.005
K_m (iii)/mM	4	9	—
K_i, Cl(ii)/mM	40	400	?
K_i, organic anions (ii)/mM	< 100	*ca.* 100	?
inhibition by metals (Cu^+, Ag^+, Hg^+, Zn^{2+})	+ +	+ + +	?
presence in:			
erythrocytes	not in dog, sheep, dolphin, chicken, shark, cattle	found in all species examined	?
epithelia	in some	in many	?
muscle	?	?	in red striated muscle and in liver of male rats

TABLE 4. PROPERTIES OF SOME INHIBITORS OF CARBONIC ANHYDRASE FROM ERYTHROCYTES, RECALCULATED FROM CARTER & PARSONS (1973)

(For one variety of CA III (A) from muscle, K_i for acetazolamide has been reported to be 240 μM (Tashian *et al.* 1980).)

inhibitor	M_r	water solubility / mM	K_i CA I	K_i CA II
acetazolamide	222	2.3	270 nM	20 nM
methazolamide	236	6.3	100	15
CL 11366	320	1.4	8	4
ethoxyzolamide	258	0.2	2	2
sulphanilamide	172	58	26 μM	2.4 μM

to be ancestral to CA I and CA II (Henderson 1980; Tashian *et al.* 1980). Some properties of the isoenzymes are shown in table 3 and of some inhibitors in table 4.

DISTRIBUTION OF CARBONIC ANHYDRASE IN EPITHELIAL TISSUES

Carbonic anhydrase has been reported to be widely distributed in epithelia, including the choroid plexus and ciliary body of mammals and the Cl^--secreting cells of fish gills. In the intestinal tract it has been described in various tissues, ranging from the salivary glands to the distal colonic mucosa, including the ruminant forestomach and the caecum of herbivores (Carter 1972; Maetz 1976).

Urinary tract

In the urinary tract the presence of the enzyme was early reported in the kidney cortex (Davenport & Wilhelmi 1941) and also in the medulla (Pollak *et al.* 1965). The cortical enzyme seems to be of the CA II variety (Carter 1972). It is of interest that as early as 1948 it was pointed out that the kidneys of herbivorous mammals have less carbonic anhydrase activity than those of carnivorous animals (van Goor 1948). In my laboratory M. J. Carter has found that the specific activity of carbonic anhydrase in adult guinea-pig kidney is not more than

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10% of that of whole blood (Carter 1972). The findings are of interest because herbivores do not usually produce an acid urine, nor does the urine usually contain much ammonia. On the basis that in the renal proximal tubule HCO_3^- 'absorption' was achieved by the secretion of protons, Walser & Mudge (1960) calculated that the uncatalysed rate of carbonate dehydration in the lumen was not fast enough to achieve equilibrium between pH, HCO_3^- and H_2CO_3. A 'disequilibrium' pH was therefore postulated to exist in the tubular lumen; this not being found, the presence of carbonic anhydrase in the brush border was postulated (Rector *et al.* 1965) and has been reported by Wistrand & Kinne (1977). Current views that HCO_3^- movements across renal brush border and peritubular membranes occur by the transfer of CO_2 and OH^- require that the anion transport inhibitor SITS (4-acetamido-4′-isothiocyano-2,2′-disulphonic stilbene), which inhibits Cl^- exchange in erythrocytes and renal tubules, also inhibits carbonic anhydrase (Frömter 1980).

With regard to the urinary bladder, that of the turtle secretes H^+ ions at the mucosal surfaces and contains cells that stain histochemically for carbonic anhydrase (Schwartz *et al.* 1972). In *Bufo marinus*, specimens obtained from Colombia usually secrete H^+ ions at the mucosal surface and in these bladders there are cells that histochemically seem to contain carbonic anhydrase. On the other hand, specimens from the Dominican Republic do not appear to acidify the urine and the bladders contain only a limited population of histochemically identifiable carbonic anhydrase-containing cells (Rosen *et al.* 1974). It is to be remembered that histochemical tests for carbonic anhydrase can be criticized (see Leder 1980); indeed, the only way to be sure that carbonic anhydrase *is* present in a tissue is to isolate the protein and to determine at least the amino acid composition.

Pancreas

Carbonic anhydrase has been reported as being present in the pancreas (van Goor 1948; Edsall & Wyman 1958) it being suggested at that time that the HCO_3^- in the exocrine secretion was derived from CO_2 in blood. The specific activities of the enzyme in dog, rat and chicken pancreas (2.5%, 0.8% and 2.2%, respectively, of the activities in whole blood, by mass) (Maren 1967; Carter 1972) are not at all high and represent levels that could be obtained by contamination with erythrocytes. Although the classical enzyme CAII seems not to be present in significant amounts, the low-specific activity, muscle-type, CAIII enzyme may be present.

Although the carbonic anhydrase activity of extracts of animal and avian pancreas is very low, the enzyme has been demonstrated histochemically by using anti-human CAII serum in the epithelium of the intralobular and interlobular ducts of human pancreas (Kumpulainen & Jalovaara 1981). However, there seem to be no reports of attempts either to assay the activity of, or to extract the enzyme from, human pancreas.

Distribution of carbonic anhydrase in the intestinal tract

My interest in carbonic anhydrase in the intestinal tract arose from experiments on salt absorption in the small intestine and colon of small mammals (Parsons 1956, 1971). These experiments provided evidence that the metabolism of carbon dioxide is closely related to the absorption of salt by the intestine. Solutions of sodium chloride, isotonic with normal extracellular fluid and buffered with $NaHCO_3$ and CO_2 (pH 7–7.5) are absorbed approximately isotonically, and at the same time changes in the acid–base composition of the intestinal

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contents are observed. In rat, dog and man, the changes found depend upon the region of the intestinal tract studied. Thus bicarbonate ions rapidly disappear from jejunal contents but are secreted into the contents of the ileum and colon during the absorption of Ringer–bicarbonate solutions. During the absorption of such solutions (25 mM bicarbonate) from the colon and ileum, the bicarbonate concentration in the luminal contents may increase to as much as 70 mM, whereas during absorption from jejunal segments, the concentration may be reduced to around 5 mM in rat, dog and man (Parsons 1971). The secretion of bicarbonate ions into rat ileum appears to require the presence of chloride ions in the intestinal lumen (Hubel 1967, 1969). The entire small intestine of the hamster, rabbit and guinea-pig appears to resemble rat ileum and colon in that bicarbonate is secreted into the luminal contents (Wilson & Kazyak 1957; Powell *et al.* 1968).

More recently, in my laboratory we have examined the unidirectional movements of Na^+ and Cl^- in both directions between the blood and lumen of the vascularly perfused small intestine and also the colon of *Rana ridibunda* (Wade 1980; Parsons & Wade 1982). The blood and the lumen fluid was buffered with HCO_3^-/CO_2. For Na^+ across the small intestine the unidirectional fluxes are large, but there is no significant net movement, whereas across the colon a net absorption of Na^+ is found. For Cl^- across the small intestine in the direction lumen to blood the flux is the same as that of Na^+, but the influx of Cl^- from blood to lumen is much less than Na^+ in the same direction or of Cl^- in the opposite direction, so that there is a net absorption of Cl^- in the small intestine. We have also found (Parsons & Wade, unpublished results from seven paired experiments) that during the absorption of Cl^- across the small intestine of these animals, vascularly perfused by the method of Parsons & Wade (1982), there is an increase in the pH of the luminal fluid corresponding to a movement into the lumen of a quantity of HCO_3^- ions (5.24 ± 0.52 μmol cm^{-2} h^{-1}) almost exactly equivalent to the Cl^- absorbed (5.09 ± 0.39 μmol cm^{-2} h^{-1}; the difference is 0.15 ± 0.18 μmol cm^{-2} h^{-1}). This absorption appears to involve a Cl^-–HCO_3^- exchange because the increase in pH is markedly inhibited by replacing the Cl^- ion in the lumen by glucuronate. The increase in pH is also inhibited by 60% by 5×10^{-4} M acetazolamide.

HCO_3^- secretion, related at least in part to Cl^- absorption and inhibited by acetazolamide, has been observed in *Amphiuma* intestine (Imon & White 1981).

In other earlier experiments in my laboratory it was found that in rat jejunum *in vivo*, replacement of the Na^+ by Mg^{2+} was associated with a net entry of HCO_3^- into the lumen, whereas in the ileum, replacement of the Na^+ by NH_4^+ revealed a net absorption of HCO_3^- (D. S. Parsons & E. C. Wills, unpublished observations). It was proposed that some of Na^+ absorbed was exchanged for protons and some of the Cl^- for HCO_3^-, the latter process being more prominent in rat ileum and colon and the former in rat jejunum (Parsons 1956, 1971, 1973).

One possibility is that in the small intestine of the rat, some of the sodium (or NH_4^+) is absorbed in exchange for hydrogen ions, which react with the bicarbonate present in the intestinal contents, releasing CO_2. This would mean that the bicarbonate was 'absorbed' as free CO_2. Supporting evidence for this was found in the values of p_{CO_2} found in the intestinal contents at different locations along the intestinal tract (table 5).

The occurrence of Na^+–proton exchange has now been observed in vesicles of brush border membranes (Murer & Hopfer 1977).

A possible role of carbonic anhydrase in bicarbonate movements together with the fact that

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TABLE 5. EQUILIBRIUM CONCENTRATIONS OF TOTAL CO_2 ($[CO_2]_e$) OBSERVED IN CONTENTS OF DOG INTESTINE ABSORBING SALINE-BICARBONATE SOLUTION, AND CALCULATED FINAL PARTIAL PRESSURES OF CO_2 (MEANS ± S.E.M.) DURING ABSORPTION OF SALINE–BICARBONATE SOLUTION BY DOG AND RAT INTESTINE

(Data of Parsons (1956) and Swallow & Code (1967).)

	$[CO_2]_e$/mM	final p_{CO_2}/mmHg†; (*n*)	
	dog	dog	rat
jejunum	9.2	46.3 ± 0.7 (57)	59.1 ± 3.6 (9)
ileum	77.4	36.1 ± 0.7 (61)	51.0 ± 3.6 (9)
colon	72.9	29.8 ± 0.5 (105)	46.5 ± 2.1 (11)

† 1 mmHg ≈ 133 Pa.

TABLE 6. APPARENT CARBONIC ANHYDRASE CONTENT OF SOME EPITHELIA

(Values are means ± s.e.m. of six determinations except where indicated. Units represent the increase in rate of hydration of CO_2 above the uncatalysed rate at 0 °C, pH 7.4, with 4 mM CO_2 and 1 mM EDTA. Data of Carter & Parsons (1971) and Carter (1971).)

	specific activity of whole mucosal homogenate / μmol s^{-1} mg^{-1} protein
guinea-pig	
stomach	13.90 ± 0.61
gall bladder	5.69 ± 0.74 (5)
jejunum	0.38 ± 0.06
ileum	0.54 ± 0.05
caecum	4.71 ± 0.40
proximal colon	9.26 ± 0.42
whole blood	4.00 ± 0.13
bovine	
rumen	1.08 (1)

exogenous CO_2 stimulates transport in the jejunum (see Parsons 1971) formed the basis for a search for the enzyme in intestinal epithelia. Davenport & Fisher (1938) in their original report described the presence of the enzyme in the mucosa of the upper small intestine.

In preliminary experiments, M. J. Carter in my laboratory detected the presence of the enzyme in homogenates of mucosae of the stomach, small intestine, caecum and colon of rat, guinea-pig and hamster. The tissues were perfused as free of blood as possible. The specific activities of the extracts of the stomach and large intestine were at least as high as that of the blood, and, as Berend (1937) had found for the gastric mucosa, it was difficult to decide whether the activity detected in the mucosa of the jejunum and ileum was due to residual contamination with erythrocytes (table 6).

By using guinea-pig intestine where the activities were highest and taking precautions to exclude from the mixtures some metals inhibitory to the enzyme, it proved possible to identify and isolate isoenzymes in pure form from some of these tissues; knowing the recovery, usually 50–60%, and the specific activities of the purified enzymes it is possible to estimate the tissue content of these enzymes. These are given in table 7 and although the estimates are approximate it is seen that, blood apart, some tissues, e.g. rumen and caecum, contain substantial quantities of the enzyme.

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TABLE 7. ESTIMATES OF QUANTITIES OF ISOENZYMES OF CARBONIC ANHYDRASE PRESENT IN SOME EPITHELIA

(CA I, low-activity variety; CA II, high-activity variety. Specific activities of pure enzyme, units per milligram of enzyme protein: CA I, 52; CA I*a* and CA I*b*, 30; CA II*a*, 2.2×10^3; CA II*b*, 1.5×10^3. Units are micromoles CO_2 catalysed per second under conditions of assay as described in table 6. Recalculated from Carter & Parsons (1972).)

	isoenzyme in mucosal protein/(mg g^{-1})			
	CA I		CA II*a*	CA II*b*
guinea-pig				
small intestine	0.3		0.3	†
caecum	16.0		2.0	0.0
proximal colon	8.0		4.3	0.0
stomach	0.0		6.2	0.3
whole blood	3.5		1.6	0.2
bovine	CA I*a*	CA I*b*		
rumen	18.2	17.4	0.0	0.0

† Enzyme present.

There are reasons for believing that isoenzymes of carbonic anhydrase are indeed present in the mucosal epithelium of the small intestine of the guinea-pig. The enzymes are unlikely to be derived entirely from the blood; the tissue was perfused and the quantity of carbonic anhydrase activity recovered from the mucosa would require a contamination of 100 mg of tissue protein with 15 mg of whole blood protein. Yet from the haemoglobin content, not more than 3 % contamination is expected. After homogenization of the tissue in such a fashion that produces haemolysis of only about 1 % of the erythrocytes, 75 % of the activity remains in the supernatant. Finally the amounts of isoenzyme CA I and CA II found in the mucosa are approximately equal, whereas in blood the amount of isoenzyme CA I is twice that of isoenzyme CA II.

POSSIBLE ROLES OF CARBONIC ANHYDRASE IN EPITHELIA

Can the presence of two isoenzymes, CA I and CA II, in the epithelia of the intestinal tract be taken to indicate separate roles for these enzymes? Thus CA II is found in the gastric mucosa and kidney cortex with very little CA I, whereas in the guinea-pig caecum and bovine rumen large quantities of CA I are found (table 7). Thus CA II seems to be associated with the secretion of H^+ ions (and the secretion of HCO_3^- in the opposite direction) and CA I with the transport or metabolism of the products of rumen and caecal fermentations; these products include short-chain fatty acids such as acetate, butyrate and propionate, and NH_4^+. Short-chain fatty acid anions have been shown to inhibit the enzymes CA I and CA II; CA I is also inhibited by Cl^- (Carter & Parsons 1972; Parsons 1973) (see table 3). Possible roles of carbonic anhydrase in the transport of NH_4^+ have been pointed out elsewhere (Parsons & Powis 1971; Carter 1972; Parsons 1973).

An important product of intraintestinal fermentation reactions is the gas CO_2, and the presence of CO_2 in the gases present in various parts of the intestinal tract has been known since early in the nineteenth century. The quantities of CO_2 present may be substantial (table 8), and a possible role of the enzyme in facilitating diffusion of CO_2 from the contents of the viscus across the epithelium into the blood has to be considered. There is no doubt that, at least in model systems, the presence of carbonic anhydrase increases the diffusion of CO_2;

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TABLE 8. CARBON DIOXIDE CONTENT OF INTESTINAL GAS AND CONCENTRATION OF CO_2 IN SOLUTION IN EQUILIBRIUM WITH THE GAS AT 37 °C

(CO_2 content is of dry gas; equilibrium concentrations rounded to nearest integer, calculated assuming a water vapour pressure of 47 Torr (*ca.* 6270 Pa) and $\alpha_{CO_2} = 0.56$ by volume.)

location	species	$[CO_2]_e$/mM	CO_2 content in intestinal gas (%)	references
rumen–reticulum				
cattle, sheep, goat		11–16	45–67	1, 2, 3
stomach	human	1–4	4–16	1, 4, 5
duodenum	dog	8–16	36–66	6
jejunum	dog	4–8	16–32	6
small intestine	human	6–10	24–40	4
ileum	human	7	28	1
caecum	human	3	12	4
colon	dog	2–8	9–34	7
colon	human	10–17	43–70	1, 4
rectum	horse	5	22	1
rectum	human	5–50	1–11	5, 7

References: 1, Pembury (1898); 2, Kleiber *et al.* (1943); 3, Dougherty (1968); 4, Magendie (1833); 5, Levitt & Bond (1970); 6, Rune & Hendricksen (1969); 7, Steggerda (1968).

it is likely that the presence of the enzyme at both boundaries of the compartment across which diffusion occurs is important for maximum effect (Schultz 1980). Although much of the enzyme present in epithelia is soluble, significant amounts of the enzyme in the colon are membrane-bound (Carter & Parsons 1971).

It is a fact that, although possible roles of carbonic anhydrase in epithelia include (i) the catalytic generation of H^+ ions and HCO_3^- ions from CO_2 and water, (ii) the facilitation of diffusion of CO_2 and (iii) involvement in the transport of anions such as Cl^- and HCO_3^- and those of short-chain fatty acids, in the metabolism of NH_4^+ and in the secretion of K^+ (Carter 1972), the functions of these enzymes in epithelial transport are not yet clearly understood.

WHEN IS CARBONIC ANHYDRASE NOT CARBONIC ANHYDRASE?

The search for an enzyme usually begins with the requirement that a catalyst is required for a named reaction; e.g. if the reaction X → Y requires a catalyst, 'X-ase', X must be obtained and tested as a substrate. But one protein can act as a catalyst for several different reactions, as an 'A-ase', 'B-ase', etc., and it may be difficult to identify the reaction of physiological importance. Thus xanthine oxidase is highly specific for certain purines, but aldehydes can also act as substrates. In the same way, isoenzymes of carbonic anhydrase can act as phosphatases and esterases. Nevertheless, whatever may prove to be the physiological substrates of the isoenzymes, the justification for calling the isoenzymes CAI, CAII and CAIII 'carbonic anhydrase' is that each is a protein of molecular mass about 30 kDa containing one atom of zinc per mole and possessing amino acid sequences that are closely related.

The effects of known inhibitors of carbonic anhydrase must be interpreted with care. The inhibition of a process by carbonic anhydrase inhibitors is a necessary but not sufficient condition to prove that the enzyme is involved. To inhibit the intracellular enzyme, the inhibitor must achieve an adequate local intracellular concentration, so that it must enter the cell,

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possibly by reacting with some transport system and, when genuine hydratase reactions are inhibited, the reaction will be slowed to that of the spontaneous rate, but not arrested. Inhibitors of carbonic anhydrase may react with other proteins, i.e. the effects may be non-specific. However, unlike some sulphonamides, acetazolamine has no effect on Cl^-–HCO_3^- exchange via the erythrocyte membrane band 3 protein (Cousin & Motais 1976).

Future work

The proof that isoenzymes of carbonic anhydrase are present in an epithelium rests ultimately upon the isolation of the Zn-containing proteins with the appropriate amino acid sequence from the tissue. A minimum requirement, rarely realized, is the demonstration that extracts of the relevant tissue increase the rate of the forward reaction of equation (1) in direct proportion to the amount of extract added. It is possible that highly specific antibodies may be useful in the identification of particular isoenzymes in tissues (e.g. of CA III in pancreas) and of the location in the cells that contain them.

Proof that catalysis of the hydration–dehydration reactions of CO_2 is required for HCO_3^-–H^+ secretory processes requires knowledge that the uncatalysed rates of hydration of CO_2 are inadequate to account for the rate of secretion. In order to calculate the rates of the uncatalysed reaction the volume of the relevant cell water is required to be known, and the identification and measurement of this volume in particular tissues is a future task. The question of whether the diffusion of CO_2 may be limiting to secretion and the role of carbonic anhydrase in the facilitation of diffusion of CO_2 requires further investigation; studies on the intracellular location will be relevant in this respect (see, for example, Schultz 1980). Finally, the search for other catalytic properties of the three known major isoenzymes including possible interrelations with NH_4^+ metabolism (Meldrum & Roughton 1933) must continue.

Work on other proteins

Just as carbonic anhydrase may have other catalytic functions of physiological importance, it is worth examining whether manifestations of carbonic anhydrase activity are to be found in other animal proteins of quite different composition from the classical carbonic anhydrase enzymes.

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Discussion

R. M. Case (*Department of Physiology, University of Manchester, U.K.*). I should like to comment on the apparent absence of carbonic anhydrase in pancreas alluded to by Dr Parsons. The problem of measuring carbonic anhydrase activity in pancreas is related to the fact that pancreatic HCO_3^- secretion is a property only of pancreatic ductal epithelium (Case *et al.* 1980) which makes up only about 4% of the total mass of the gland (Bolender 1974). To overcome this problem, we have taken avantage of the observation that feeding rats a copper-deficient diet supplemented with copper-chelating agents causes total acinar cell atrophy, leaving ductal tissue intact (Fölsch & Creutzfeldt 1977; Smith *et al.* 1982*b*). We have estimated carbonic anhydrase activity in supernatants from pancreatic homogenates of normal and copper-deficient rats by measuring the catalysed liberation of $^{14}CO_2$ from $H^{14}CO_3^-$ (Case *et al.* 1980; Smith *et al.* 1982*a*). There is a sevenfold enhancement of activity in ductal tissue over that in whole pancreas (9.5 ± 1.2 compared with 1.3 ± 0.3 i.u. mg^{-1} DNA; means $\pm$ s.e.m.).

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