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Anion Transport in the Colon

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Anion transport in the colon

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The principal anions transported by colonic epithelium are Cl^-, HCO_3^- and organic anions (OA^-), particularly acetate, butyrate and pyruvate, these last being formed by microbial degradation of carbohydrate. In the normal absorptive rat colon, Cl^- is transported from lumen to plasma both by the transcellular and paracellular pathways. The transcellular route appears to depend on amiloride-insensitive coupling of Na^+–Cl^- at the mucosal (apical) membrane, the Na^+ electrochemical gradient energizing Cl^- uptake. Intraluminal [HCO_3^-] rises as Cl^- is absorbed, and a mucosal Cl^-–HCO_3^- exchange carrier has been postulated. In some species (and in distal colon of the rat when sodium-depleted), the putative Na^+–Cl^- carrier is absent so that Cl^- absorption then depends largely on the paracellular electrochemical gradient. Absorption of OA^- is independent of the transepithelial p.d., is associated with HCO_3^- secretion and is considerably reduced by acetazolamide. In the absence of Cl^-, OA^- supports Na^+ absorption but does not depend on it continuing unchanged when the latter is blocked. Colonic epithelium can become secretory and an example of this state is congenital chloridorrhoea in which an elevated transepithelial p.d. is associated with excessive Cl^- secretion. Here, it appears that the Na^+–Cl^- and Cl^-–HCO_3^- carriers are lost and Cl^- conductance of the mucosal membrane substantially increased. The transepithelial uphill movements of Cl^- or HCO_3^- in the absorptive and secretory colon appear to depend on coupling to other ionic flows, and there seems to be no need to postulate active transport of these ions.

Introduction

As the gut contents pass through the colon a considerable fall of Na^+ concentration and a rise of K^+ concentration in the faecal fluid occurs. The functions of the colonic epithelium that produce these changes have attracted most attention. The original observations of Curran & Schwartz (1960) showed that Na^+ was absorbed against the electrochemical gradient, an active Na^+ transport system being postulated: they gave little attention to anions but concluded that Cl^- absorption was entirely passive, which since the blood side was positively charged with respect to the lumen, depended largely on the transepithelial electrical p.d. Although, since then, most studies of colon have been towards elucidating the cation transport mechanisms, various observations have indicated that the anion transport mechanisms are of some complexity and are influential on cation movements. Particularly important in regard to anion transport is the fact that the colon is one of the natural fermentation chambers of an animal. Indeed, in many, for example the carnivores, omnivores and some hervibores which have only simple stomachs, the large intestine provides the only fermentation region. Although in many species, the absorption of organic anions (OA^-), principally acetate, butyrate and propionate, produced by microbial fermentation contributes little to overall nutrition; in others, for example the rabbit and the pony, the contribution is substantial (reviewed by Wrong *et al.* 1981).

In all species, however, the colon plays a major part in the conservation of inorganic ions and water. The overall changes are well established. The transit of the gut contents from

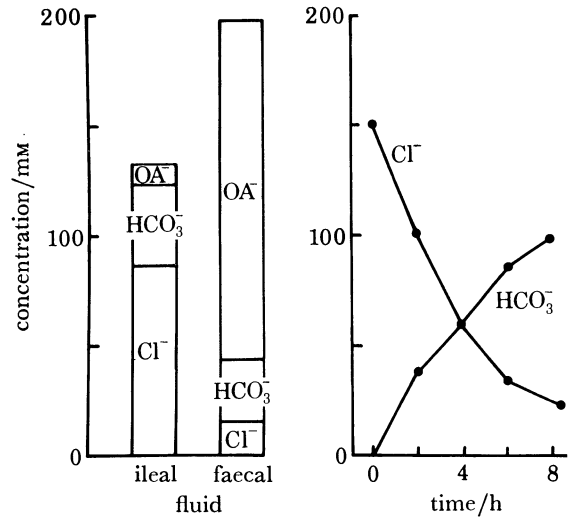

FIGURE 1. (*a*) Anion composition of ileal effluent and faeces in man (Wrong *et al.* 1981). (*b*) Change of composition of isotonic NaCl solution during prolonged perfusion of human colon.

terminal ileum is associated with a decrease in Cl^- concentration, a considerable increase in OA^- and usually a small rise in HCO_3^- concentration in the faecal water (figure 1). Studies on cleansed intestine produce a somewhat different picture because microbial OA^- generation is eliminated: Cl^- concentration falls markedly with HCO_3^- concentration rising reciprocally. The mechanisms concerned in producing these changes will be reviewed in this brief survey but it first needs to be emphasized that there are considerable species and other variations, which are unfortunately often ignored when generalizations are made. These variations are demonstrated in species differences, in differences between various segments of the large intestine, and the effects of nutritional state, hormonal influences and age (Edmonds 1967; Bentley & Smith 1975; Fromm & Hegel 1978). Pathological processes and various intraluminal agents, for example bile salts, can also profoundly modify epithelial function. Studies *in vitro* do not, of course, eliminate the problem because the epithelium can be modified by its history. There is therefore plenty of opportunity for discrepant results to arise between observers, although such anomalies may simply reflect modulation of similar basic mechanisms by more or less undefined influences. In the present account, I shall mainly be considering our observations on the distal segment of rat colon but shall add a few additional comments to indicate some observed segmental, species and other variations.

TRANSCELLULAR AND PARACELLULAR PATHWAYS

As with other epithelia, the movement of ions across colonic epithelium can proceed by two principal routes: by a transcellular pathway with the ions crossing the plasma membranes and cell substance, and by a paracellular or shunt route with the ions bypassing the cells and moving, it is believed, through the intercellular spaces and tight junctions. We have previously described a method (Edmonds & Smith 1979) which from observations of the blood and secretory curves of changing activity of radioisotopic tracers after the administration of an intravenous bolus, allows estimation of the paracellular component of the plasma to lumen (J^{i}_{sm}). We have applied this method to examine cation and anion movements across rat colonic epithelium. In regard to the anions, the results show that normally the Cl^- flow into the lumen

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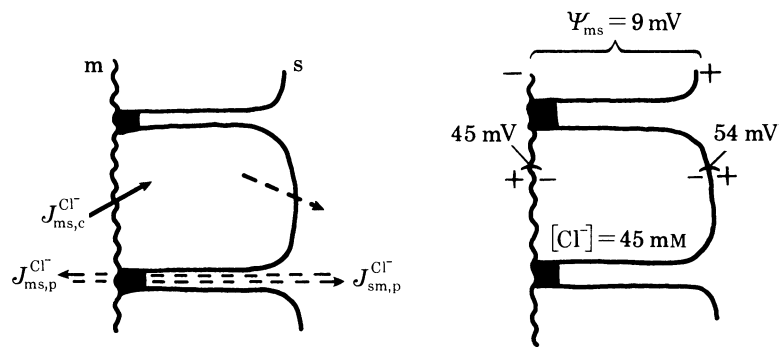

FIGURE 2. (*a*) Transcellular and paracellular pathways for Cl^-. The flux values for normal rat were estimated as described in the text. The interrupted lines indicate a passive flux; m refers to mucosal or lumen side; s refers to serosal or blood side. (*b*) Electrical gradients (mean values) obtained by microelectrode measurements in rat distal colon (Edmonds & Nielsen 1968).

completely, or nearly so, is accounted for by Cl^- movement through the paracellular path, with the mean transit time of the ions being $20 + 9$ (s.d.) s. This value was similar to that obtained, for example, with I^- and Na^+, while full data analysis gave spectra of transit times distributed as unimodal functions about the mean. These observations were consistent with a simple diffusion path length of about 100–150 μm, in good agreement with the sort of distance that the ions have to traverse in passing from the subepithelial capillaries into the luminal perfusion fluid. Absorption of anion can also be measured in these experiments by observing the net change in luminal solution composition. Thus lumen plasma flux (J^i_{ms}) can be determined. The flux J^i_{ms} has two components, one passive and paracellular, $J^i_{ms,p}$ and the other transcellular, $J^i_{ms,c}$:

$$J^i_{ms} = J^i_{ms,p} + J^i_{ms,c}. \quad (1)$$

The component $J^i_{ms,p}$ is estimated from the observed values of J^i_{sm}, the transepithelial p.d., ψ_{ms}, the activities of the solutions of the lumen and blood, a_m and a_s respectively, using the flux ratio equation (Ussing 1952)

$$J^i_{ms,p} = J^i_{sm}(a_m/a_s) \exp(Z_i F\psi_{ms}/RT),$$

where F, R, T and Z_i have their usual significance. There in this is estimation the assumption that the ion movement is unaffected by ion–ion or by ion–solvent interactions. For colonic paracellular Cl^- movement these assumptions are justified on the basis of various other studies. From these measurements and calculations carried out for Cl^-, the scheme shown in figure 2 for normal rat distal colon emerges. The mean values for distal colon of four rats when lumen Cl^- concentration was 100 mM were: $J^{Cl^-}_{sm,p} = 72$, $J^{Cl^-}_{ms,p} = 133$ and $J^{Cl^-}_{ms,c} = 70$ nmol min^{-1} cm^2.

TRANSCELLULAR AND PARACELLULAR Cl^- MOVEMENTS

As noted already, the plasma–lumen flux is entirely paracellular and so, in this set of experiments, was most of the opposite flux, $J^{Cl^-}_{ms}$. However, about one-third of the flux was transcellular. The electrochemical gradients have been previously determined for the epithelial cells of rat colon (Edmonds & Nielsen 1968). Similar values have been obtained for rabbit distal colon (Schultz *et al.* 1977). The flow of Cl^- from the lumen into the epithelial cells across the mucosal (apical) membrane is therefore much against the electrochemical gradient (figure 2*b*). This Cl^-

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movement therefore cannot be simply passive and we must seek other processes to provide energy for the uptake of Cl^- by the cells. One such process, discussed in more detail later, is by exchange with HCO_3^- produced by the cells, but in addition Na^+ movement from the lumen into the cells appears to provide a major driving force for the Cl^- uptake.

Several independent lines of evidence support this notion. First, there is the finding with rat colonic mucosa *in vitro* that short-circuit current was usually considerably less than the net Na^+ flow, whereas removal of Cl^- from the lumen solution markedly decreased the latter although leaving the short-circuit current little changed (Binder & Rawlins 1973). Second, experiments involving infusion of NaCl solutions into the colon by way of implanted cannulae showed that

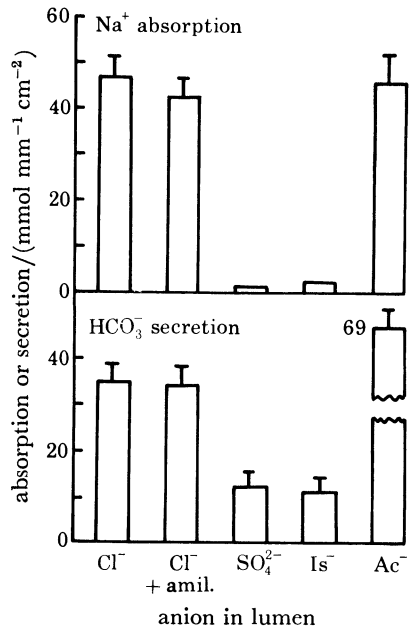

FIGURE 3. Effect of substituting various anions within lumen on Na^+ absorption and HCO_3^- secretion. Na^+ and anion concentration was 100 mM throughout, amiloride was used at a concentration of 10^{-3} M. Is^-, isethionate; Ac^-, acetate (means ± s.e.m.).

absorption increased severalfold without significant change in transepithelial p.d. (Edmonds & Thompson 1980). Third, replacement of the Cl^- in the lumen solution by SO_4^- or by isethionate substantially reduced transcellular Na^+ absorption although the transepithelial p.d. was little affected (figure 3). Finally, Na^+ and Cl^- absorption by normal rat colon was little if at all affected by amiloride (Wills *et al.* 1980; Edmonds 1981) a finding quite unlike that in epithelia in which Na^+ enters the cells apparently by a simple diffusional step. As will be discussed later, in the rat and in other species, colonic Na^+ and Cl^- absorption may become partly or completely amiloride-sensitive under some circumstances.

The stoichiometry of the apparently linked Na^+–Cl^- absorption has not been adequately worked out, but some indication can be obtained from our results. To do this, the data obtained for Cl^- were related to comparable data coming from experiments in which ^{22}Na was given in the intravenous bolus to estimate the paracellular Na^+ component. Studies with the use of saline of Na^+ concentration 100 mM gave mean flux values of

$$J^{Na^+}_{sm,\,p} = 90, \quad J^{Na^+}_{ms,\,p} = 35, \quad J^{Na^+}_{ms,\,c} = 131 \text{ nmol min}^{-1} \text{ cm}^2.$$

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The crude figures therefore suggest a ratio of about 2:1 for the mucosal membrane Na^+–Cl^- coupling. There is also, however, the Cl^-–HCO_3^- exchange to be taken into account; if this is 1:1 exchange (see below), 4:1 is the more likely figure for the Na^+–Cl^- linkage. Even from this fairly crude estimate, it would appear that the putative mucosal membrane carrier is charged so that the transmembrane electrical gradient, which depends on the epithelial Na^+ pump (Schultz 1972), contributes the major force for Cl^- as well as Na^+ absorption. The coupling between the pump and the mucosal entry step is then preserved and Cl^- entry to the cells, although 'uphill' is essentially dependent on Na^+ movement and Na^+ pump activity.

A form of coupling between Na^+ and Cl^- entry to the epithelial cells, as outlined, offers the simplest solution. It is phenomenological, nothing is known about the molecular basis, and moreover it is not a unique explanation. Other possibilities such as some form of complex linkage involving Na^+–H^+, Cl^-–HCO_3^- exchanges across the mucosal membrane cannot be excluded (Argenzio & Whipp 1981).

Amiloride sensitivity induced by dietary Na^+ deficiency or aldosterone

The scheme above has been described for rat colon, but Schultz *et al.* (1977) found that rabbit distal colon (studied *in vitro*) was normally completely sensitive to amiloride; other segments of the rabbit's rather complex colon were not examined. In piglets and in the hen colon and coprodeum, change from amiloride-insensitive and apparently Na^+-anion-coupled absorption to an amiloride-sensitive system has been described, in the former associated with increase in age, in the latter with sodium-deficient diet (Cremaschi *et al.* 1979; Thomas *et al.* 1979). In the rat, too, amiloride sensitivity can be induced by NaCl deficiency or by aldosterone administration: only partial sensitivity can be produced in the proximal part but a complete change occurs distally (Will *et al.* 1981; Edmonds 1981). While the absorption of Cl^- is unaffected by amiloride in the normal rat, once rats have been treated for several days with aldosterone or are on a restricted sodium intake, amiloride markedly interferes with Cl^- absorption (figure 4). It therefore appears that in the aldosterone-treated rats, Cl^- absorption by the distal colon has become dependent on the electrical gradient and on Cl^-–HCO_3^- exchange alone, the Na^+–Cl^- coupling being lost.

Coupling of Cl–HCO_3^- movement

Perfusion studies in a variety of species have long since shown that lumen solutions develop concentrations of HCO_3^- considerably in excess of those expected from the transepithelial electrochemical gradient (D'Agostino *et al.* 1953; Wrong 1971): this could result from net movement of either H^+ from the lumen or OH^- or HCO_3^- in the opposite direction. The results from rat colon led Parsons (1956) to suggest the presence of a Cl^-–HCO_3^- exchange mechanism. Subsequent studies have supported this view (Phillips & Schmalz 1970; Binder & Rawlins 1973). The appearance of HCO_3^- is relatively specific, the substitution of other anions for chloride in general appearing to eliminate the effect (figure 3), while the amount of apparent HCO_3^- secretion reaches maximal values at relatively low intraluminal Cl^- concentration (figure 5). Moreover, Schultz *et al.* (1977) employing *in vitro* methods with rabbit distal colon, in which tissue Na^+ transport can be completely blocked and the transepithelial p.d. abolished

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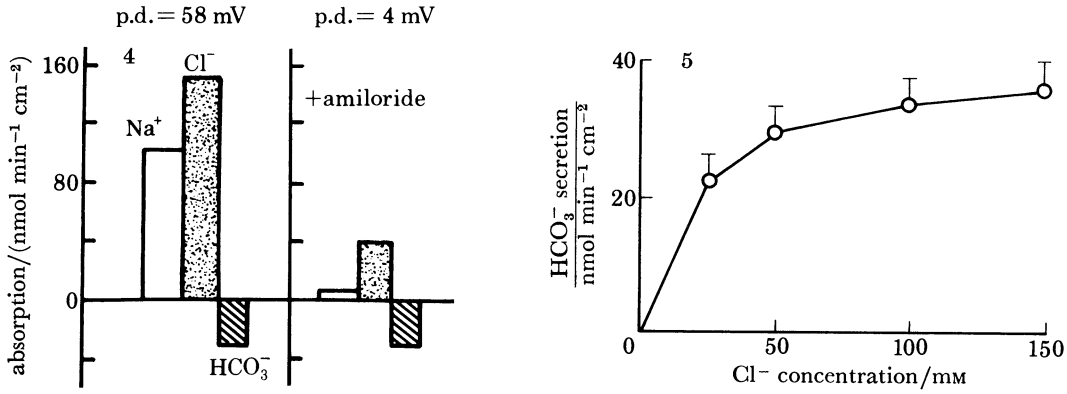

FIGURE 4. In a sodium-depleted rat amiloride (10^{-4} M) in the lumen nearly abolished the transepithelial p.d., inhibited Na^+ absorption and considerably reduced Cl^- absorption. Means obtained from three measurements. The solution in the lumen was 100 mM NaCl with mannitol to render it isotonic.

FIGURE 5. Apparent secretion rate of HCO_3^- observed in the distal colon of six normal rats during perfusion with solutions of varying Cl^- concentration. The solutions also contained Na^+ as cation and mannitol to render them isotonic. (data from Edmonds 1967).

by amiloride, found that the Cl^-–HCO_3^- substitution was electrically silent, suggesting 1 : 1 exchange. Consistent with this, considerable changes of p.d. produced by amiloride in sodium-depleted animals did not affect HCO_3^- secretion (figure 4). There is therefore a body of evidence consistent with the notion, developed in some detail in small intestinal studies by Turnberg *et al.* (1970), that a Cl^-–HCO_3^- exchange occurs probably through a common carrier in the mucosal membrane of the epithelial cells. The exchange could be driven by the electrochemical gradient for HCO_3^- across the luminal membrane. Carbonic anhydrase is located in colonic epithelial cells (Carter 1972) and may well be involved in production of HCO_3^- ions from precursor CO_2 produced by metabolic activity of the cells. The carbonic anhydrase inhibitor acetazolamide considerably reduces the apparent secretion of HCO_3^-, but because Na^+ and Cl^- movements are also influenced the interpretation of this observation remains uncertain (Parsons 1956; Phillips & Schmalz 1970).

Absorption of organic anions

The importance of OA^- in colonic anion transport mechanisms has tended to be ignored in biophysical studies, but large amounts of OA^- are generated naturally in the colon by bacterial activity, especially from plant fibre of dietary origin (Elsden *et al.* 1946). As a consequence in many species examined, OA^- concentrations in the large bowel often exceed 100 mM, corresponding to 75% or more of the total anion present (reviewed in Wrong *et al.* 1981). It is now well established that OA^- is rapidly absorbed by colonic epithelium (Ruppin *et al.* 1980; Argenzio *et al.* 1977; McNeil *et al.* 1978). In the rat distal colon, studies with acetate as the OA^- showed that absorption was proportional to the concentration in the lumen, did not depend on the presence of Na^+ within the lumen (despite the fact that Na^+ absorption itself is well supported by OA^-; see figure 3) nor did it appear to depend on the transepithelial p.d. (figure 6). The absorption of OA^- was independent of Cl^- concentration within the lumen but was associated with the appearance of HCO_3^- (figure 3). Umesaki *et al.* (1979) interpreted the latter finding as indicating stimulation of HCO_3^- secretion by OA^- within the lumen. The alternative

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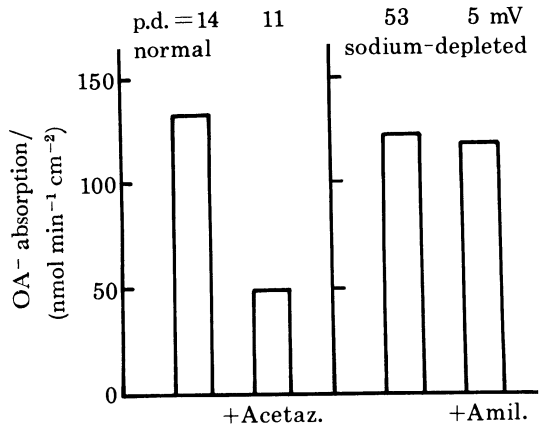

FIGURE 6. Effect of acetazolamide in a normal and of amiloride in a sodium-depleted rat on organic anion (OA^-) absorption. The test solution contained 50 mM Na acetate (including [^{14}C]acetate) rendered isotonic with mannitol. Note that 10^{-4} M amiloride almost eliminated the transepithelial p.d. and Na^+ absorption in the sodium-depleted rat without affecting OA^- absorption.

explanation is that it simply results from the diffusion of undissociated acid from the lumen, thus:

$$RCOO^- + CO_2 + H_2O \rightarrow RCOOH + HCO_3^-.$$

Consistent with this notion is the observed reduction of intraluminal p_{CO_2} in perfusion experiments (Ruppin *et al.* 1980), as well as other observations suggesting the significance of undissociated acid absorption. Thus absorption has been shown to be increased when pH is decreased, and in order of lipid solubility of the undissociated acid, i.e. butyrate > propionate > acetate (Leng 1978). Moreover, acetazolamide administration (10 mg intravenously) substantially reduced OA^- absorption (figure 6), an observation consistent with the possibility that production and secretion of H^+ by the epithelium may play a part in undissociated acid formation and absorption. In this respect, it may be relevant that in guinea-pig at least (Carter 1972) much larger quantities of carbonic anhydrase are present in the proximal colon, which is the region that absorbs the bulk of OA^- (as well as Na^+ and water), than in the distal part. At present, however, the precise relation between these various observations remains largely speculative and considerably more evidence is needed to define the mechanism of OA^- absorption in the colon.

ANION SECRETION BY THE COLON

Anion secretion by colonic epithelium ordinarily appears to be confined to HCO_3^-. But under various special circumstances, secretion of Cl^- has been observed (table 1). In particular in rat colon, Cl^- secretion has been provoked by exposure of the mucosal side of the epithelium to certain hydroxy fatty acids (Racusen & Binder 1979), to cyclic 3,5-AMP (cAMP) and to some Ca^{2+} ionophores (Frizzell, Welsh & Smith 1981). In addition, ricinoleate was found to increase the epithelial cAMP content. Both cAMP and Ca^{2+} may therefore be involved in a common pathway by which the secretion is mediated. Some of the agents employed in promoting secretion undoubtedly damage the epithelium and possibly produce secretion in this way (Chadwick *et al.* 1976; Nell *et al.* 1976). However, anion secretion frequently does not appear to involve damage as indicated at least morphologically or in effects on other characteristic functions such as Na^+ pump activity and hormonal responses. A primary increase in Cl^-

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TABLE 1. STIMULATION OF COLONIC SECRETION

(1) neurogenic factors, e.g. acetylcholine (Browning *et al.* 1978)
(2) gastrointestinal hormones, e.g. vasoactive intestinal peptide (VIP)
(3) secretogogues, e.g. cAMP, Ca^{2+} ionophores
(4) congenital abnormality, e.g. chloridorrhoea
(5) acquired pathology, e.g. colitis
(In addition to anions, water and cations are usually also secreted.)

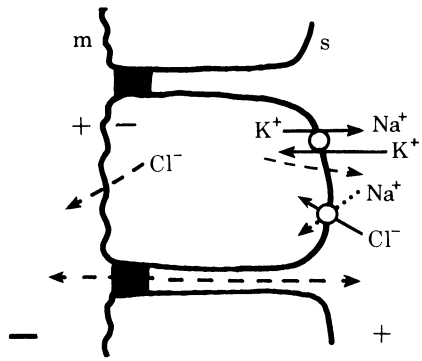

FIGURE 7. Postulated Cl^- secretion mechanism that depends on enhanced Cl^- conductance of the mucosal membrane. On account of various experimental observations, Frizzell *et al* (1981) also proposed the existence of Na^+–Cl^- coupled entry on the basolateral side operant in the secretory state.

conductance of the mucosal membrane has therefore been suggested (Naftalin & Simmons 1979; Frizzell *et al.* 1981). Cl^- movement into the lumen from the cells then passively follows the electrochemical gradient across the mucosal membrane. The transepithelial p.d. will be increased and as a consequence there is increased Na^+ movement into the lumen through the paracellular pathway. Thus although an apparent uphill movement of Cl^- across the epithelium is observed, there is not necessarily any active anion secretion because the energy for the Cl^- movement derives from the electrochemical gradients, which depend on the basolateral Na^+ pump. It is usually assumed that one set of epithelial cells are involved in absorptive and, under some conditions, secretory activity. We have, however, no unequivocal evidence on this point and possibly secretion of Cl^- results from the activity of a different set of cells which, usually in abeyance, can be provoked by various stimuli (Browning *et al.* 1978).

One particularly interesting but rare congenital abnormality (congenital chloridorrhoea) mimics these experiments. In this condition relatively high concentrations of Cl^- develop in faecal fluid, exceeding even the total of Na^+ and K^+ concentrations (Evanson & Stanbury 1965). We carried out a series of studies on one individual with this condition, a young man who had severe diarrhoea since birth. He passed fluid stools which showed the characteristic ionic composition: a number of collections made over several weeks gave mean values for $[Cl^-]$ of 110 mM and of $(Na^+ + K^+)$ of 95 mM in the faecal water. Studies of epithelial function in which absorption and secretion were examined by using various solutions placed in contact with the epithelium showed significant abnormalities (table 2). We found that the transepithelial p.d. of his colon was considerably greater than we had observed in normal individuals and there was moreover an apparent active Cl^- secretion by the colon, the net Cl^- movement into the lumen occurring against the transepithelial electrical gradient. The amount of HCO_3^- produced was very small. The observations strongly suggest that in congenital chloridorrhoea

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TABLE 2. TRANSEPITHELIAL P.D., Na^+, K^+, Cl^- AND HCO_3^- NET FLUXES OF DISTAL COLON IN AN INDIVIDUAL (M.C.) WITH CONGENITAL CHLORIDORRHOEA (MEAN ± S.E.M.)

	p.d. mV	net flux (nmol min^{-1} cm^{-2}) Na^+	K^+	Cl^-	HCO_3^-
M.C.†	45	67	−164	−84	−13
normal (5)	28	338	−51	372	−85
	±3	±24	±5	±29	±7

† Calculated from data of the study of Pearson *et al.* (1974). Results of M.C. are means of two measurements with the use of a lumen solution having concentrations of Na^+, 75; K^+, 25; Cl^-, 100 nM (mannitol added for isotonicity). The negative sign indicates net movement towards the lumen.

there is an abnormality of the mucosal membrane of the epithelial cells such that Cl^- conductance is much enhanced while the putative Cl^-–HCO_3^- exchange carrier is lost. More detailed study of the epithelia in such patients is needed and may be considerably revealing as to the molecular mechanisms involved.

CONCLUSION

Organic anions, Cl^- and HCO_3^- are the principal anions transported across colonic epithelium. Several mechanisms are involved and these show variations due to segment, species, diet and other factors. The epithelium is electrically polarized but the transepithelial electrochemical gradients cannot adequately explain the anion movements observed. Describing the movements in terms of the paracellular and cellular pathways allows us to evolve hypotheses about the way the ions traverse the epithelium and about the electrochemical forces involved. These can be tested under various conditions, and when the epithelium is in its 'absorptive' or 'secretory' state. In the 'absorptive' state it is suggested that the transcellular route requires at least some linkage of Na^+–Cl^- and of Cl^-–HCO_3^- at the mucosal (apical) membrane. Exactly how OA^- crosses the membrane remains uncertain but may depend on the formation of undissociated acid. In the 'secretory' state, increased Cl^- conductance of the mucosal membrane seems of particular importance although epithelial damage producing a very 'leaky' epithelium may sometimes also be important. There is, however, no evidence that special anion pump have to be postulated: appropriate carriers together with the extant electrochemical gradients produced by operation of the Na^+ pump appear sufficient to account for the observed movements.

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