Ion Transport in Isolated Rabbit Ileum

III. *Chloride fluxes*

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ABSTRACT Unidirectional Cl fluxes across *in vitro* segments of rabbit ileum have been determined both in the absence and in the presence of an electrochemical potential gradient. The results indicate that Cl transport in this preparation can be attributed to purely passive forces uninfluenced by solvent drag or exchange diffusion. Furthermore, on the basis of this and previous studies, it has been demonstrated that the sum of the partial ionic conductances of Na and Cl accounts for at least 90 per cent of the total tissue conductance.

Previous studies of ion transport across short-circuited *in vitro* segments of the terminal ileum of the rabbit have demonstrated that Na is actively transported from mucosa to serosa and that the short-circuit current and the transmural potential difference may be attributed entirely to this process (1, 2). These observations are, however, insufficient to exclude the presence of active Cl transport, which, by being linked to the movement of either another anion or a cation other than Na, does not contribute to the short-circuit current. The possible presence of such a mechanism is suggested by the results of other investigators who have convincingly demonstrated both active Cl absorption (3) and active Cl secretion (4) by *in vivo* preparations of mammalian small intestine.

The present study is concerned with the determination of unidirectional Cl fluxes across isolated rabbit ileum both in the presence and absence of an electrochemical potential gradient and was undertaken in order to directly define the mechanism of Cl transport in this system. It will be shown that Cl movements may be attributed to passive transport processes uninfluenced by solvent drag, exchange diffusion, or single file diffusion, and that at least 90 per cent of the total tissue conductance can be accounted for by the combined partial ionic conductances of Na and Cl.

METHODS

Cl fluxes were determined using Cl^{36} which was assayed in a Tricarb liquid scintillation spectrometer. All other methods and procedures have been previously described in detail (1).

RESULTS AND DISCUSSION

The results of a series of experiments in which the mucosa-to-serosa (Φ_{ms}^{Cl}) and serosa-to-mucosa (Φ_{sm}^{Cl}) Cl fluxes were determined in the absence of a

TABLE I

Cl FLUXES, TISSUE CONDUCTANCE, AND SHORT-CIRCUIT CURRENT IN ISOLATED RABBIT ILEUM*

$\{\psi_s - \psi_m\}$‡	No. of observations	Φ_{ms}^{C}	Φ_{sm}^{C}	Φ_{net}^{Cl}§	$\Phi_{ms}^{Cl}/\Phi_{sm}^{Cl}$	ξ‖	G	I_{sc}
mv			*μmol/cm² hr.*				*mmhos/cm²*	*μmol/cm² hr.*
				Glucose absent				
0	30	7.0±0.2	7.3±0.2	−0.3±0.3	0.97±0.05	1.00	18.3±0.4	3.1±0.1
−25	20	4.2±0.2	10.5±0.5	−6.3±0.6	0.40±0.03	0.39	16.7±1.3	—
				Glucose present (10 mM)				
0	18	6.9±0.3	6.7±0.3	0.2±0.4	1.03±0.06	1.00	17.2±0.4	4.3±0.2

* All errors are standard errors of the mean.
‡ The transmural PD corrected for fluid resistance.
§ $\Phi_{net}^{Cl} = \Phi_{ms}^{Cl} - \Phi_{sm}^{Cl}$.
‖ $\xi = \exp [F(\psi_s - \psi_m)/RT]$.

transmural electrochemical potential gradient (*i.e.*, in the short-circuited state)[1] are given in Table I. The average values of Φ_{ms}^{Cl} and Φ_{sm}^{Cl} do not differ significantly, and in the absence of an electrochemical potential gradient the net Cl flux (Φ_{net}^{Cl}) does not differ significantly from zero. Furthermore, the unidirectional Cl fluxes are not influenced by the presence (3 experiments) or absence (5 experiments) of glucose in the perfusion medium whereas the short-circuit current (I_{sc}) (column 9) and the rate of active Na transport are markedly increased by the presence of this actively transported sugar (1, 2). Finally, the values given in Table I represent the average values of samples obtained during a 1 hour sampling period which commenced 15 to 20 minutes after the onset of perfusion. The unidirectional Cl fluxes gradually increased during this 1 hour interval from an average value of 5.9 μmol/cm² hr., during the first 10 minutes, to 8.3 μmol/cm² hr., during the final 10 minutes. However,

[1] In the present experiments correction was made for fluid resistance between the agar bridge tips, as discussed previously (1), so that all PDs reported refer to the actual transmural PD.

the two opposing unidirectional Cl fluxes did not differ significantly during any 10 minute sampling period. During the same period the average tissue conductance (see below) increased from 16.4 mmhos/cm² to 19.0 mmhos/cm².

The results thus far presented support the conclusion that Cl is not actively transported by this preparation. These results are, however, insufficient to rule out the presence of carrier-mediated exchange diffusion (5), a process which might well contribute significantly to the flux in both directions but would not contribute to the partial ionic conductance of Cl. Hogben (6) has shown that a significant fraction of the unidirectional Cl flux across frog gastric mucosa may be attributed to exchange diffusion, and Diamond (7) has arrived at a similar conclusion with respect to Br^{82} fluxes across fish gall bladder.

In order to determine whether a significant carrier-mediated exchange diffusion component is present in this preparation, unidirectional Cl fluxes were determined in the presence of an imposed transmural electrochemical potential gradient obtained by "clamping" the tissue at an arbitrarily selected PD. Ussing (8) has shown that if the two opposing passive unidirectional ionic fluxes are influenced exclusively by their respective electrochemical potential gradients (*i.e.*, no solvent drag, exchange diffusion, or single file diffusion), the following relationship should obtain:

$$\Phi^{Cl}_{ms}/\Phi^{Cl}_{sm} = (a^{Cl}_m/a^{Cl}_s) \exp [F(\psi_s - \psi_m)/RT] \quad (1)$$

where the subscripts m and s refer to the mucosal and serosal solutions respectively; ψ is the electrical potential; a^{Cl} is the chloride activity; and F, R, and T have their usual meanings. In all of the present studies $(a^{Cl}_m/a^{Cl}_s) = 1$.

The results of a series of experiments in which Φ^{Cl}_{sm} and Φ^{Cl}_{ms} were determined when the transmural PD was "clamped" at -25 mv (all PD's refer to the serosal surface with respect to the mucosal surface) are given in Table I. The good agreement between the observed flux ratio (column 6) and that predicted by equation (1) (column 7) is consistent with the conclusion that Cl movements in distal rabbit ileum are *entirely* passive with no evidence for the influence of solvent drag, exchange diffusion, or single file diffusion.

Tissue Conductance

We have previously demonstrated that rabbit ileum behaves as a linear resistor over the range -30 to $+50$ mv (1). Although the determination of tissue conductance using direct current measurements is subject to error due to polarization, this error is relatively small in the present, low resistance system. For this reason in these studies the total tissue conductance, G, was determined from the I_{sc} per unit area (corrected for fluid resistance) and the spontaneous PD.

As shown in Table I, G both in the presence and absence of glucose did not

differ significantly and averaged 17.8 mmhos/cm². Since the Cl fluxes are passive, the partial conductance of Cl in mmhos/cm², G_{Cl}, is almost exactly numerically equal to the unidirectional flux of Cl, in the short-circuited state, given in μmol/cm² hr. (see Equation 1.3.15, reference 9). Thus, during the 1 hour sampling period G_{Cl} averaged 7.1 mmhos/cm² and accounted for approximately 40 per cent of the tissue conductance.

Linderholm has shown that the partial ionic conductance of an actively transported ion can be calculated from the rate of active transport in the short-circuited state, and the "active transport potential" (9). For Na transport by distal rabbit ileum, the partial Na conductance in the short-circuited state is given by

$$G_{Na} = 26.8\ (\Phi_{net}^{Na}/E_{Na}) \qquad (2)$$

where Φ_{net}^{Na} is in μmol/cm² hr., G_{Na} is in units of mmhos/cm², and E_{Na} is the active transport potential of Na in millivolts. Using previously published values for Φ_{net}^{Na} and E_{Na} (1), the values of G_{Na} both in the presence and absence of glucose do not differ significantly, and average 9.0 mmhos/cm². These values were obtained during experiments of 35 minutes' duration which commenced after a 15 to 20 minute equilibration period. During a comparable sampling period in the present experiments G_{Cl} averaged 6.3 mmhos/cm² and G averaged 16.9 mmhos/cm². Since the sum of G_{Na} and G_{Cl} accounts for 91 per cent of the total tissue conductance, the extent to which other ionic species may contribute to the transmural current is markedly restricted. The relative constancy of the I_{sc}, PD, G, and the fluxes of both Na and Cl encountered in these preparations justifies the above conclusion which is based on a comparison of two different series of experiments.

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