PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 1220–1224 (1974)

Electrolyte Transport Across the Mouse Small Intestine[1] (37985)

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Reports from this laboratory (1, 2) and others (3–7) have demonstrated that the mammalian intestine provides a useful experimental model for studies on the transport mechanisms for electrolytes and nonelectrolytes. The animals used in these studies were mainly rats, rabbits, hamsters, and guinea pigs. The use of mouse intestine as a model system for such studies has not been previously explored.

It is generally agreed that the measured ion fluxes and short-circuit current across the mammalian intestine vary greatly with the thickness of the intestine, presumably because of the presence of several layers in addition to the mucosal membrane. Therefore, data obtained with stripped intestinal mucosal membrane is preferable to that obtained with an intact intestine (4, 6, 7). Since mouse intestine is much thinner than that of mammals previously studied, one wonders would the ion fluxes across the thin intact mouse intestine correspond closely to those observed in the stripped mammalian intestinal mucosa. If so, the mouse intestine could provide a useful and simple tool in future studies on drug effects on intestinal transport systems.

Methods. Albino Swiss–Webster mice, weighing an average of 30–40 g were used. They were kept in a constant-temperature room with food and water *ad lib.* for 7 days or longer after purchase. The mice were killed by decapitation, and the abdomen was opened. A section of jejunum–ileum was removed and opened along the line of mesenteric attachment, rinsed with Ringer solution and mounted in a lucite Ussing chamber having an aperture area of 1.3 cm^2. Mammalian Ringer or other isotonic solution was used as bathing fluid. Seven milliliters of bathing solution was pipetted into each of the two chambers separated by the intestinal membrane. The bathing solution was continuously stirred and oxygenated by bubbling with 95% O_2–5% CO_2 gas. The chamber was kept in a 37°C incubator throughout the experiment. The composition of each bathing solution is given in Table I. Calomel electrodes were placed close to each side of the membrane for potential difference (PD) measurement. Current was sent through two $PbSO_4$ electrodes to zero out the PD and recorded in an Esterline-Angus recorder. The design of the Ussing chamber and the details of the electrical measurements have been described previously (8, 9).

Radioactive ^{22}Na and ^{36}Cl were used to measure the sodium and chloride fluxes across the short-circuited intestine. Bathing solutions from both sides were sampled at half-hour intervals and were counted in duplicate in automatic well-scintillation and liquid scintillation counters. Standard ^{22}Na and ^{36}Cl were counted simultaneously with the samples. The ratios of counting of these standard solutions by the two counters were then used to determine the amount of ^{22}Na and ^{36}Cl in the collected samples. Electrolyte fluxes and short-circuit current (I_{sc}) are expressed in microequivalents (μEq) per square centimeter per hour.

Results. Electrical properties of mouse intestine. When the mouse intestine was

[1] This work was supported in part by National Science Foundation Grant GB 27495-A1 and Public Health Service Grant AM-02217-14.

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TABLE I. Ionic Composition of the Bathing Solutions.[a]

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	SO_4^{2-}	PO_4^{3-}	Mannitol	Acetate
NaCl Ringer with HCO_3	141	5	1.1	1.2	115	25	1.2	1.6	0	5
HCO_3^- free	141	5	1.1	1.2	140	0	1.2	1.6	0	5
Na_2SO_4 Ringer	141	5	1.1	1.2	0	25	60.2	1.6	69	5
Choline Cl Ringer	0	8	1.1	1.2	115	25	1.2	1.6	0	5

[a] Expressed as millimoles per liter.

bathed with bicarbonate–NaCl Ringer solution, a potential difference (PD) ranging from 1–5 mV was observed, with serosa electropositive to mucosa. The resistance ranged from 80–150 ohms. The PD remained stable for at least an hour. In 12 experiments the average PD was 1.47 ± 0.17 (mean ± SE) and the average I_{sc} was 15.8 ± 1.56 μA. Replacement of the bicarbonate NaCl Ringer solution with bicarbonate–Na_2SO_4 Ringer resulted in decreases in both PD and I_{sc} across the intestine. On replacement of NaCl Ringer solution with choline chloride Ringer solution, the PD and I_{sc} decreased nearly to zero. This effect was reversible by returning to NaCl Ringer solution. Figure 1 presents a typical experiment demonstrating these electrical properties of mouse jejunum. The addition of D-glucose to the bathing solution, resulted in an increase in measured PD and I_{sc}. In 12 experiments in the presence of D-glucose 5.5 mM the average PD was 3.81 ± 0.17 and the average I_{sc} was 43.4 ± 3.73. On subsequent addition of phlorizin (0.5 mM), the augmented PD and I_{sc} returned to the

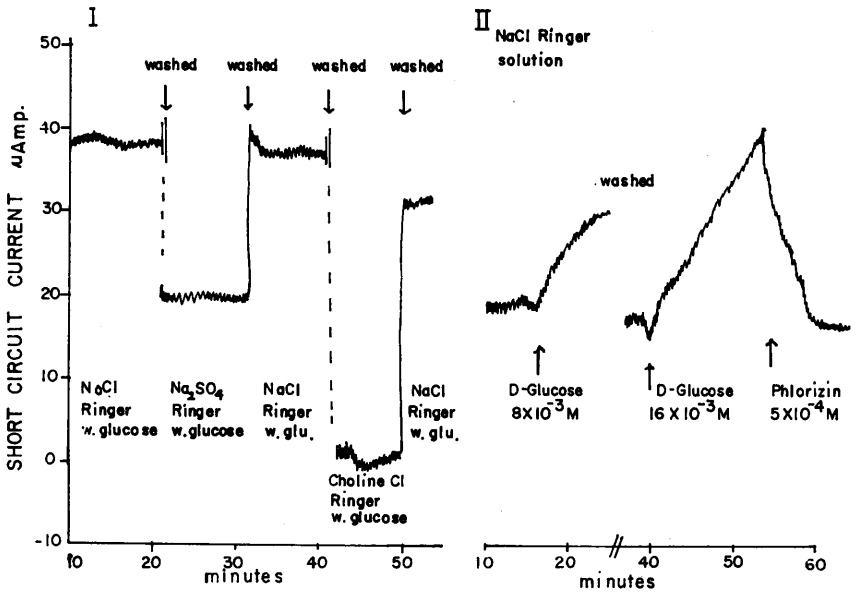

FIG. 1. Electrical properties of mouse intestine. Ordinate is short-circuit current (I_{sc}) orientated with respect to serosal side. Abscissa is the time of measurement. The intestinal chambers were kept at constant temperature, 37°C, throughout the experiment. I. Effect of bathing fluids on I_{sc} across the intestine. In each fluid replacement period both chambers were rinsed with the same bathing solution twice and the recorder was stopped for 1–2 min. II. Effect of D-glucose and phlorizin on I_{sc} measurement.

original levels. Figure 1b presents a typical experiment on the effect of phlorizin on the electrical properties of the mouse intestine.

Isotopic measurement of fluxes across the mouse intestine. Table II summarizes the results of simultaneous measurement of ^{22}Na and ^{36}Cl fluxes under various conditions. In all experiments presented here two adjacent jejunal–ileal sections were used to simultaneously measure the mucosal-to-serosal (J_{ms}) and serosal-to-mucosal fluxes (J_{sm}). It can be seen that when the intestine was bathed in a bicarbonate–NaCl solution, there was a net Na absorption and a net Cl secretion. The net of these two ion fluxes was far greater than that calculated from I_{sc}. Glucose increased the ion fluxes in both directions, but with a greatest effects on the J_{ms} of Na and J_{sm} of Cl ion, resulting in an increase of short-circuit current and PD.

When Na_2SO_4 Ringer solution was used as bathing fluid, both J_{ms} and J_{sm} of Na ion were smaller than those observed in NaCl Ringer resulting in a roughly 50% reduction in net Na absorption. In choline Cl Ringer solution, both PD and I_{sc} were practically reduced to zero, but there was still a measurable Cl ion outflux, averaging 1.8 μEq per hr-cm^2.

Omission of bicarbonate buffer from the bathing solution did not affect the electrical measurements. However, the J_{ms} of Na ion was greatly reduced in the absence of bicarbonate, resulting in a net flux of zero for this ion. Although both the J_{ms} and J_{sm} fluxes of Cl ion were reduced in HCO_3-free NaCl Ringer solution, there was still a net Cl secretion, averaging -2.18 μEq/hr-cm^2, and the calculated residual J_{net}^R was nearly abolished. This is shown in Fig. 2.

Discussion. Data presented here demonstrate that the electrical properties of unstripped mouse intestine are similar to those observed with the unstripped intestine of other mammals (3–5) and different from those reported for fish intestine (8, 9). For example, the present experiments show that a PD exists across the mouse intestine orientated such that the serosa is electro-

TABLE II. Isotopic Measurement of Fluxes Across the Mouse Intestine.[a]

Bathing solution	n	J_{ms}	J_{sm}	J_{net}	I_{sc}	J_{net}^{R}[b]
Bicarbonate-buffered NaCl Ringer (without glucose)						
^{22}Na	18	10.24 ± 0.90[c]	9.60 ± 1.40	+0.66 ± 0.31	0.44 ± 0.03	−1.30
^{36}Cl	18	6.36 ± 1.0	7.44 ± 1.80	−1.08 ± 0.20		
With glucose						
^{22}Na	18	16.65 ± 0.53	13.90 ± 0.50	+2.76 ± 0.20	1.31 ± 0.05	−6.17
^{36}Cl	18	7.14 ± 0.58	11.86 ± 0.42	−4.72 ± 0.18		
HCO_3^--free NaCl Ringer with glucose						
^{22}Na	18	6.77 ± 0.46	7.00 ± 0.81	−0.23 ± 0.15	1.70 ± 0.18	−0.25
^{36}Cl	16	5.23 ± 0.28	7.41 ± 0.40	−2.18 ± 0.26		
Na_2SO_4 Ringer with glucose						
^{22}Na	18	8.90 ± 0.50	7.60 ± 0.58	1.96 ± 0.40	0.54 ± 0.08	−1.52
Choline Cl Ringer with glucose						
^{36}Cl	12	8.30 ± 0.48	9.42 ± 0.64	−1.26 ± 0.20	~0	−1.26

[a] Expressed as μEq. hr^{-1} cm^{-2}.
[b] $J_{net}^R = I_{sc} - (J_{net}^{Na} - J_{net}^{Cl})$.
[c] Mean ± SE.

positive to the mucosa. The PD and I_{sc} were reduced by replacement of NaCl Ringer with Na_2SO_4 Ringer solution and dropped practically to zero after replacement of the NaCl Ringer solution with choline Cl Ringer solution (Fig. 1). These electrical properties indicate there is a transmural transport of cation which is at least partially chloride dependent. Addition of D-glucose to the bathing solution, either NaCl Ringer or Na_2SO_4 Ringer solution, augmented both PD and I_{sc} across the intestinal membrane and this stimulating effect was abolished in the presence of 0.5 m*M* of phlorizin.

The isotopic flux measurements of the present studies confirmed the transport of Na ion across the unstripped mouse intestine against an electrochemical gradient by a glucose stimulating system. Net Cl secretion, occurring both in the presence and absence of Na, was also demonstrated in these studies.

Intestinal transport of Cl ion has been demonstrated in fish (8, 9) and mammalian (4, 6, 7) with the direction of transport in the latter varying with the species studied. For example, Field *et al.* (4) demonstrated a net mucosal-to-serosal transport (absorption) of Na and Cl ions in the stripped short-circuited rabbit ileal mucosa, while Powell, Binder, and Curran (6, 7) found net Na absorption and net Cl secretion under similar conditions in the guinea pig ileum, similar to that found with the unstripped mouse intestine.

In the guinea pig experiments Powell *et al.* (6) found the I_{sc} to be greater than the sum of J_{net}^{Na} and J_{net}^{Cl} and suggested that the residual flux was due to bicarbonate secretion. In the experiments with mouse intestine the observed I_{sc} was small, being less than the sum of J_{net}^{Na} and J_{net}^{Cl} and yielding a negative net residual flux, indicating a residual net anion absorption or cation secretion. The measured I_{sc}, J_{net}^{Na}, and J_{net}^{Cl}, the calculated J_{net}^{R} and the electrolyte fluxes possibly responsible for the residual flux are illustrated in Fig. 3. The negative net residual flux in HCO_3–NaCl Ringer ($J_{net}^{R} = 6.7$ μEq/hr-cm^2) could be accounted for by (1) bicarbonate absorption, either directly or via a Na-H exchange process, (2) active hydrogen secretion, or (3) secretion of other cations such as K^+ or Ca^{2+}. The present observation that both J_{net}^{Na} and J_{net}^{R} were reduced to practically zero in HCO_3-free NaCl Ringer suggests that either bicarbonate absorption or H^+ secretion is responsible for

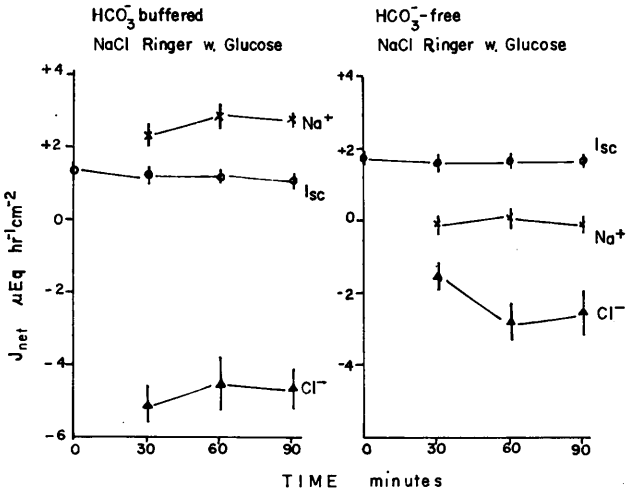

FIG. 2. Effect of HCO_3^- on Na and Cl fluxes across the mouse intestine. Ordinate is calculated J_{net} for Na, Cl and I_{sc}. Each point is the average of six to eight experiments ± one standard deviation. Abscissa is the time of measurement. After the electrical properties of the intestine became stable, radioactive ^{22}Na and ^{36}Cl were added into the chamber, this was taken as zero time.

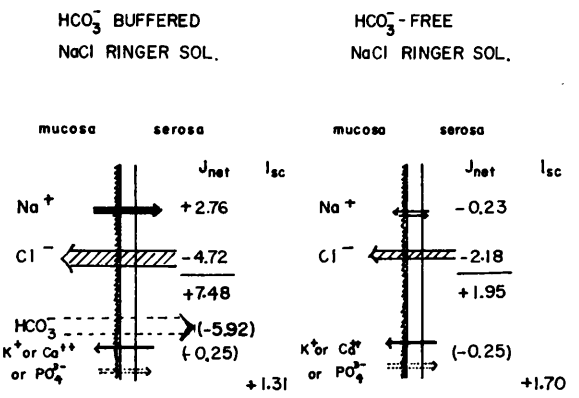

FIG. 3. A diagrammatic representation of ion fluxes across the mouse intestine. The numbers in parentheses indicate the calculated residual fluxes, J_{net}^{R}.

the residual flux in HCO_3^- containing Ringer solution. These findings further indicate that net Na absorption is dependent on HCO_3^- absorption or H^+ secretion, the former via a Na–H exchange mechanism seeming most likely according to the studies of Turnberg *et al.* (10) in human jejunum. These workers, who also found that Na transport against an electrochemical gradient in the human jejunum in the presence but not in the absence of HCO_3^-, concluded that the relation between the transport of these electrolytes is best explained by a Na–H exchange process.

Summary. The electrical and electrolyte transport properties of nonstripped mouse intestine were investigated using the Ussing chamber technique. In HCO_3–NaCl Ringer solution a PD of 1.47 ± 0.17 mV and I_{sc} of 15.8 ± 1.56 μA existed across the intestine, orientated such that the serosa was electropositive to the mucosa. Radioisotope flux measurements demonstrated net Na absorption and Cl secretion. The I_{sc} was less than the sum of net Na and Cl fluxes ($J_{net}^{Na} + J_{net}^{Cl}$), yielding a negative net residual flux (J_{net}^{R}), indication of a simultaneous anion absorption or cation secretion. Glucose augmented $J_{net}^{Na} + J_{net}^{Cl}$ and increased the PD and I_{sc}. In the absence of bicarbonate, the J_{net}^{Na} and J_{net}^{R} were reduced to practically zero, suggesting that either HCO_3^- absorption or H^+ secretion is responsible for the residual flux observed in HCO_3^- Ringer. These findings also indicate that Na absorption is HCO_3^- dependent.

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Received June 8, 1973. P.S.E.B.M., 1974, Vol. 145.