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Intra-and Extracellular Gradients of Electrical Potential and Ion Activities of the Epithelial Cells of the Rabbit Ileum In vivo Recorded by Microelectrodes

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Phil. Trans. R. Soc. Lond. B 1975 **271**, 277-281
doi: 10.1098/rstb.1975.0052

Phil. Trans. R. Soc. Lond. B. **71**, 277–281 (1975) [277]
Printed in Great Britain

Intra- and extracellular gradients of electrical potential and ion activities of the epithelial cells of the rabbit ileum *in vivo* recorded by microelectrodes

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The epithelial cells of the rabbit ileum are about 40 μm long and have a diameter of roughly 5 μm. They are closely packed in a columnar fashion with their mucosal ends facing the lumen of the intestine and their serosal ends abutting the basement membrane and facing the underlying capillaries, lymphatics and connective tissue. The inner wall of the intestine is coated with a layer of mucopolysacharides, the mucus layer. When Krebs solution or similar solutions are placed in the lumen it is well known (e.g. the review by Edmonds 1970) that the solution is transported into the underlying tissues. During this transport the sodium activity in the gut lumen remains constant, the chloride activity decreases and the bicarbonate activity increases. However, the relationship of the intra- to the extracellular environment of each individual cell during this transport is only little understood.

In recent years the methods applied in the study of the state of the cells during this transport have mostly been of a type that requires preparation of the tissue *in vitro*, e.g. usage of flame-photometry and Ussing-chambers. In case of the intestine of warm-blooded animals such a choice of experimental conditions is unfortunate for several reasons: (*a*) The transport is no longer into the blood or lymphatic drainage, (*b*) the epithelial cells have to be oxygenated from their luminal ends which *in vivo* are normally at a very low oxygen concentration (Crompton, Silver & Shrimpton 1965) and (*c*) in some experiments the physiological parameters of the tissue change during the experiment (e.g. Powell, Binder & Curran 1973). However, the use of microelectrodes allows an *in vivo* approach since the tissue can be left in its natural environment during the measurements. Furthermore liquid ion exchanges and ion-sensitive glasses developed over the past few years have led to the construction of microelectrodes selective to various ions (Hinke 1959; Thomas 1970; Walker 1971), so that the state of cells in terms of intra- and extracellular electrical potential and ion activities can be determined. Thus intracellular sodium and potassium has been recorded in the epithelial cells of the bullfrog small intestine *in vitro* (Lee & Armstrong 1972).

The purpose of this work was first to construct an *in vivo* model in which the epithelial cell layer of the rabbit ileum could be studied by microelectrodes and secondly to determine the intra and extracellular electrical potential and ion activities in these cells when the lumen of the intestine was filled with various salt solutions.

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Methods

In White New Zealand rabbits anaesthetized with sodium pentobarbitone an incision 5 cm long was made in the midline of the abdominal wall into the abdominal cavity. With the rabbit lying on one side a loop of 20 cm of the terminal ileum was taken out and supported on a perspex plate which was warmed to 38 °C. A slit 6 mm long was made through the upper side of the serosa of the intestine so that a Perspex tube (5 mm outer diameter and wall thickness of 1 mm) could be passed vertically through the slit and pressed against the opposite mucosal side of the gut, the serosal side of which was resting on the perspex plate. The position of the tube was so adjusted that the blood supply was maintained to the tissue as observed through the heated plexiglass plate. In this position the seal between the tube and the tissue prevented leakage of the mucosal solution (usually 145 mM NaCl, 5 mM KCl buffered to pH 7.8 with phosphate buffer, or Krebs solution) which was placed in the tube. Microelectrodes were advanced into the tissue via the tube. If peristaltic movements disturbed the microelectrode recordings, incisions were made in the muscle layers. If the tissue was kept moist and warm the preparation could be used for at least 2–3 h. During this period the tissue looked healthy, as determined from microscopical histological examination.

We used three types of double-barrelled microelectrodes: one barrel (the reference barrel) always recorded the electrical potential and the other was either used for (*a*) iontophoretic staining of the recording site or to record either (*b*) potassium activity or (*c*) chloride activity when filled with the appropriate liquid ion exchanger (Corning 477317 potassium exchanger or 477315 chloride exchanger). Staining was done either by lithium carmine (Villegas 1962) or by Procion Yellow MHRS (Stretton & Kravitz 1968). The ion selective microelectrodes were prepared as described by Zeuthen, Hiam & Silver (1974). The reference barrel was filled with either 2 M KCl or 2 M NaCl and in a few experiments with 1 M NH_4NO_3. The impedance of this barrel was about 7–10 MΩ when measured in 2 M KCl with a tip potential that varied less than 3 mV in different isotonic solutions of a strength of 150 mM mixed from NaCl and KCl. The electrodes had a total tip diameter of less than 0.6 μm.

Results

When the tip of an electrode is advanced from the lumen through the layer of epithelial cells and into the underlying tissues a series of increasingly negative steps in potential were usually recorded by the reference barrel (figure 1). This was finally followed by an abrupt shift towards small negative values (at B in figure 1). The electrode was advanced at a rate of about 2 μm/s until a sudden negative step was recorded when the advance was stopped for about 10–100 s to record the potential. If stain was deposited after every second or third change in potential as shown at points S in figure 1 we could identify spots separated by at least one cell diameter in the epithelial cell layer in subsequent histological sections. If we used Procion Yellow the spots had the shape and dimension of the epithelial cells. Thus each negative step corresponded to the intracellular electrical potential of an epithelial cell. If stain was deposited at a place after the potential had returned to the near zero values (after B in figure 1) it was found to be located in the tissues below the basement membrane. These step-wise profiles of electrical potential were also present in other experimental situations. (*a*) When the electrode was advanced from the serosal side, through the epithelial cells and into the lumen, then a series

of decreasingly negative potentials were recorded. (*b*) If the solution at the lumen side was stirred the potentials recorded were unchanged. (*c*) If in the mucosal solution sulphate ions were substituted for chloride ions, or if the potassium activity was increased to 50 mM (sodium activity decreased to 100 mM).

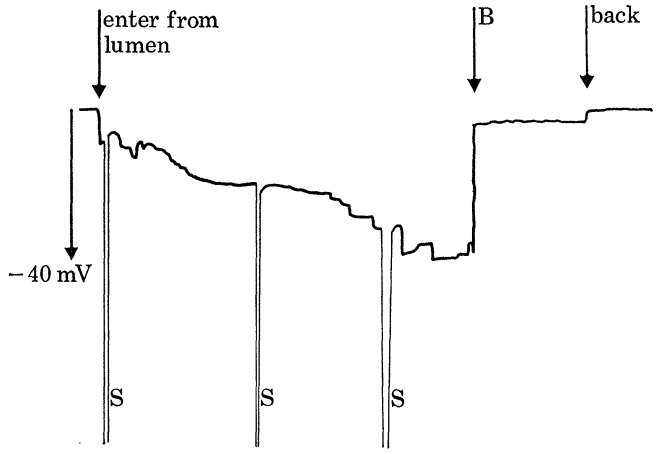

FIGURE 1. The electrical potential as recorded by a microelectrode advancing through rabbit ileum epithelial cells. The electrode was advanced at a speed of about 2 μm/s until a stepwise negative increase in potential was recorded when the advance was arrested for 10–100 s. Each plateau of electrical potential is recorded intracellularly as ascertained by iontophoretic staining (at S). When the tip of the electrode has passed out of the epithelial cell layer at B it was pulled back into the lumen of the gut.

With 2 M KCl in the reference barrel the first negative potential that was recorded when the electrode was advanced into the epithelial cells from the luminal side was -5.0 ± 0.29 mV ($\pm$s.e.m., 9 recordings from 6 animals) while the final negative potential was -34.4 ± 2.7 mV which was recorded just before the electrode entered the underlying tissues. With 2 M NaCl in the barrel the first negative potential was -6.6 ± 1.0 mV (7 recordings from 4 animals) and the final was -43.9 ± 3.8 mV. However the difference between the potentials obtained with the two types of electrodes was not significant: Student's *t*-test; $P < 0.1$ in the luminal end of the cell, $P < 0.06$ in the distal end. When the tips of the electrodes were deep to the epithelial cell layer both types of probe recorded -6.5 ± 4.9 mV (23 recordings from 6 animals). In a few experiments where the reference barrel was filled with 1 M NH_4NO_3 we obtained potentials similar to those above.

The intracellular potassium activity recorded with ion-selective double-barrelled electrodes also depended on the depth of penetration: each stepwise decrease in electrical potential was associated with a stepwise increase in the potassium activity. If the reference barrel was filled with 2 M KCl the measured potassium activity increased from about 50 mM in the mucosal end of the cell to a maximum average of 160 mM (sometimes as high as 200 mM) near the basement membrane before entering into the underlying tissue where we obtained as a mean 11 mM. If the reference barrel was filled with 2 M NaCl similar results were obtained, although the recorded potassium activity tended to decline after its initial stepwise increase.

In about half of 92 animals the electrode passed through a identifiable layer of up to 50 μm thickness, probably the mucus layer, before it started to record the stepwise increasing negative potentials. In this layer potassium activity increased towards the tissue, i.e. it had the same activity as the luminal solution (5 mM) in its outer parts and increased to typically 25–30 mM adjacent to the cell surface. The electrical potential was a few millivolts positive in this layer.

Finally in two animals the chloride activity and electrical potential was recorded across the epithelial cell layers. Each increasingly negative step in potential was associated with a stepwise decrease in chloride activity: a maximum of roughly 80 mM was obtained intracellularly close to the lumen and a minimum of about 10 mM was obtained near the basement membrane. Extracellularly, deep to the basement membrane a hyperosmolarity of roughly 300 mM was obtained. In these experiments the reference barrel was filled with 2 M KCl.

Discussion

Our findings can be explained if there exists intracellularly along the length of each epithelial cell a gradient of electrical potential, of potassium activity and of chloride activity: the cells are depolarized at the end facing the lumen, with a low membrane potential, low potassium activity and probably a high chloride activity while they become gradually more polarized towards their serosal ends. If the measurements of the chloride activity are confirmed by other experiments with solutions in the reference barrel which do not contain chloride, or with electrodes of a smaller tip diameter, then the extracellular environment around the serosal end of the cells is hypertonic to that of blood serum. The existence of hyperosmolar areas is in agreement with current theories on water transport (Curran 1960; Diamond 1964). The possibility of intracellular gradient of ion activities in these tissues has been hypothesized by Lindemann & Pring (1969) in relation to standing gradients of solutes in the extracellular spaces (Diamond & Bossert 1967).

When the luminal potassium activity is low (5 mM) the difference in chemical potential of the potassium ions between the inside of the cells and the underlying tissues is about 70 mV (61 mV $\times$ lg{160 mM/11 mM}) compared to the electrical potential difference (about -30 mV). This suggests that potassium here is actively transported into the cell. Across the mucosal part of the cell membrane the chemical potential difference is about 10 mV (61 mV $\times$ lg {50 mM/25 mM}) which, compared to the electrical potential difference -6 mV, indicates a non-equilibrium distribution of potassium across this part of the cell membrane; the gradient of potassium in the mucus layer shows that potassium is transported into the lumen when the intra-luminal potassium activity is low which is in agreement with the finding that naturally occurring fluids in the terminal ileum contain 20–25 mM potassium (Edmonds 1970).

The question now arises how these gradients of activities and potentials are maintained. They are not a simple result of the net transport of potassium into the lumen (or chloride out of the lumen), since they were also present when the intra luminal activity of potassium was increased to 50 mM (or the chloride in the lumen exchanged for sulphate), and must therefore be an inherent property of the organization of the epithelial cells. Furthermore the results stress the importance of the mucus layer for the transport to and from the lumen; thus it is well known that in *enteritis* when the mucus layer is altered by inflammatory processes, there may be severe loss of potassium into the lumen.

With the potential of the lumen taken as 0 mV we recorded on average a slightly negative potential behind the epithelial cell layer whereas most epithelial tissues (see review by Schultz & Curran 1968) have a slightly positive potential when recorded between two large electrodes placed in solutions bathing the lumen and the serosal side of the intestine. However the two measurements are not directly compatible since we did not attempt to determine whether the

potentials recorded by the microelectrodes were recorded close to or inside capillaries, lymphatics or other tissues. Thus the mean value of the potentials inside the capillaries might still be positive.

Our data of the intracellular environment are not in disagreement with the data of others. An intracellular potential of −36 mV was recorded by Rose & Schultz (1971), and −10 mV to −15 mV by Field & Curran (unpublished observations, quoted by Schultz & Curran (1968)) from the rabbit ileum *in vitro*, and in the jejunum of the rat Barry & Eggenton (1972) obtained about −10 mV; in these studies however no attempt was made to correlate the recording site with the microanatomy of the tissue; it is therefore possible that Rose & Schultz systematically recorded closer to the serosal end of the cell than Barry & Eggenton. Schultz, Fuisz & Curran (1966) report an average intracellular potassium concentration of 140 mM and Frizzell *et al.* (1973) report an average chloride activity of about 67 mM; these values are only slightly higher than the averaged values we obtained from our study. Lee & Armstrong (1972) obtained a potassium activity of 85 mM in the bullfrog small intestine.

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