Biochimica et Biophysica Acta, 413 (1975) 104–115

BBA 77108

EFFECTS OF POTASSIUM IONS AND SODIUM IONS ON MEMBRANE POTENTIAL OF EPITHELIAL CELLS IN RAT DUODENUM

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(Received April 28th, 1975)

SUMMARY

1. Mucosal and serosal membrane potentials (V_m and V_s) of epithelial cells in rat duodenum were recorded together with the transmural potential differences (PD_t).

2. The value of V_m in rat duodenum at 37 °C was about −53 mV, being considerably greater than the values reported hitherto for the small intestine of various species.

3 When Cl^- in the mucosal medium was partially replaced with SO_4^{2-} at fixed mucosal Na^+ and K^+ concentrations ($[Na^+]_m$ and $[K^+]_m$), the membrane potential was scarcely affected in the steady state several minutes after replacement, whereas marked changes in the potential were observed with varying $[K^+]_m$ or $[Na^+]_m$.

4 As the mucosal K^+ concentration increased at constant $[Na^+]_m$, V_m was gradually decreased (depolarization), together with the increase in PD_t Such a change in V_m caused by varying $[K^+]_m$ obeys Nernst's equation in the range of $[K^+]_m$ higher than about 60 mM

5 At constant $[K^+]_m$, an increase in $[Na^+]_m$ also caused the decrease of V_m for the lower $[K^+]_m$ region, whereas V_m was not affected by such changes in $[Na^+]_m$ in the range of $[K^+]_m$ higher than approx 60 mM.

6. The values of P_{Na}/P_K were obtained from the modified Goldman equation under an appropriate assumption. The ratio of the permeability coefficients markedly increases from zero to approx 0 07 with a decrease in $[K^+]_m$.

INTRODUCTION

Electrophysiological studies of the epithelial cells in the small intestine are of great importance for analyzing properties of the electrically inexcitable membrane which possesses active ion and solute transport mechanisms Using rat duodenum in vitro, we found that the membrane potential of the epithelial cell was considerably greater (around − 53 mV) under normal conditions than that reported hitherto in the small intestine [1–9] Several investigators reported that the membrane potential in small intestine is dependent on the ionic environment [1, 3, 7] To acquire detailed

knowledge of the ionic basis of membrane properties, we attempted to analyze the effect of K^+ and Na^+ concentration changes over a wider range on the potential profiles

METHODS

Tissue preparation

After a fast of 24–48 h except for free access to water, adult rats were anesthetized with ether and the abdominal cavity was opened by a midline incision. The duodenum was cut into lengths of about 2 cm, and using a syringe the lumen was rinsed clean with fresh buffered saline until it was free of intestinal contents in the presence of an intact blood supply. As soon as the intestine had been cut open along the mesenteric border, the sheet of duodenal mucosa was mounted between the two halves of the Lucite chamber as shown in Fig. 1. To obtain high membrane potential it was critically important to maintain intact blood supply just prior to mounting the preparations

Solutions

A phosphate-buffered saline was used as the control medium throughout the present study. In order to change K^+, Na^+ and Cl^- concentrations in the mucosal fluid, modified buffered salines were prepared with the same osmolarity and pH (7 3±0.1) as the control medium. Compositions of these modified solutions, as well as the control, are illustrated in Table I Desired concentrations of K^+, Na^+ and Cl^- were obtained by mixing these solutions appropriately.

Electrical potential measurements

The transmural potential difference (PD_t) was measured with a high-input impedance preamplifier (Nihon Koden MZ-3B) connected to the mucosal and serosal fluids by calomel cells and salt bridges consisting of polyethylene tubing (1 3 mm outside diameter) filled with 1 % agar/3 M KCl. The membrane potential was mea-

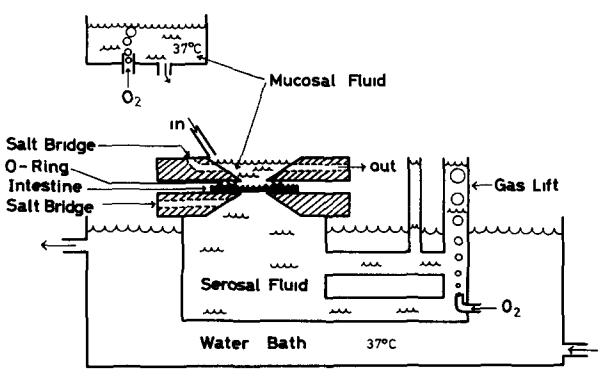

Fig 1 Schematic view of bathing chamber The area of the window between the two halves of the chamber was 0 28 cm^2 The mucosal fluid was perfused at about 1 ml/min by gravity from a reservoir, and the serosal fluid was recirculated by means of oxygen gas lifts Mucosal and serosal fluids were maintained at 37 °C, and bubbled with 100 % O_2

TABLE I

IONIC COMPOSITION OF MODIFIED BUFFERED SALINES AND THE CONTROL PHOSPHATE BUFFERED SALINE

Each of the modified buffered salines had the same pH (7 3±0 1), tonicity and ionic strength (except SO_4^{2-} medium) as the control medium The ionic strength of SO_4^{2-} medium was a little greater (about 10 %) than the other modified buffered salines or the control saline All solutions contained 20 mM mannitol In addition, an appropriate amount of mannitol (about 100 5 mM) was added to the Tris medium to keep the total tonicity constant Concentrations are mM

	Control medium	SO_4^{2-} medium	Na^+ medium	K^+ medium	Tris medium
K^+	4 2	4 2	—	147 2	4 2
Na^+	143 0	143 0	147 2	—	—
Cl^-	132 5	5 5	132 5	132 5	146 0
Mg^{2+}	0 5	0 5	0 5	0 5	0 5
Ca^{2+}	0 9	0 9	0 9	0 9	0 9
$Tris^+$	—	—	—	—	139 0
SO_4^{2-}	—	63 5	—	—	—
HPO_4^{2-}	8 0	8 0	8 0	8 0	—
$H_2PO_4^-$	1 5	1 5	1 5	1 5	—

sured by the microelectrode technique under visual control with a binocular microscope The mucosal membrane potential (V_m) and the serosal membrane potential (V_s) were taken to be the potential of the recording microelectrode with respect to the reference electrode in the mucosal and serosal fluids, respectively

Recording microelectrodes filled with filtered 3 M KCl solution were prepared by the glass fiber method [10] Glass tubing and glass fibers were pretreated with a strong acid in order to obtain microelectrodes of low tip potentials [11]. Their resistances and tip potentials ranged from 10 to 30 MΩ (mean 16 MΩ) and from 0 to −4 7 mV (mean −1.7 mV), respectively. The reference electrode was a micropipette

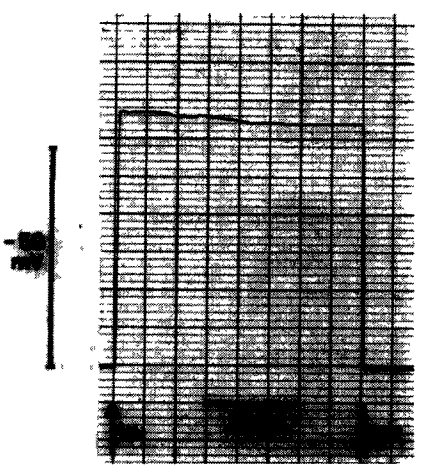

Fig 2 Recording of the membrane potential in rat duodenum Upward and downward arrows indicate the time of entry and exit of the recording microelectrode, respectively

filled with agar/3 M KCl, the tip of which had been broken off. An appropriate pair of Ag/AgCl electrodes for connection to the preamplifier was selected so that the difference in their junction potentials in 3 M KCl was practically zero

The criteria for an acceptable impalement were: (1) a sharp jump to peak voltage when the electrode penetrated into the cell; (2) the maintenance of a stable PD within ± 2.5 mV for at least 10 s; (3) an abrupt return to the original baseline when the electrode was removed from the cell. A typical recording is shown in Fig. 2 If the potential did not return to its original baseline upon withdrawal from the cell, the data were rejected, regardless of the magnitude.

Since the initial peak was taken as a measured value and the exchange of the mucosal fluid brought about the movement of villi, we could not continuously follow up changes in the potential in a test solution of one and the same cell. All data presented are, therefore, the values obtained in different successively punctured cells. Such a procedure resulted in a fluctuation of data, so statistical analyses were applied. When a test solution of non-physiological ion composition was employed, it was replaced by physiological saline within 10 min.

RESULTS

Potential profiles in physiological media

The transmural potential difference (PD_t) measured in duodenum was 2.1 ± 0.09 (S.E.) mV ($n = 160$). The mucosal membrane potential (V_m) measured on 644 epithelial cells in duodenum ranged, as shown in Fig. 3, from -40 mV to -74 mV; their mean value being -53.0 ± 0.20 mV. These V_m values are larger than the values reported previously [1–9].

After the measurement of V_m and PD_t with respect to the mucosal fluid at ground, the serosal fluid was grounded by a switching device and the serosal membrane potential (V_s) was recorded while the microelectrode was still inside the same cell The mean values measured in such an experiment were -50.42 ± 2.17 mV ($n = 9$) for V_m, -53.11 ± 2.57 mV ($n = 9$) for V_s, and 2.67 ± 0.47 mV ($n = 9$) for PD_t. The mean PD_t calculated as the algebraic difference between V_m and V_s is 2.70 ± 0.50 mV

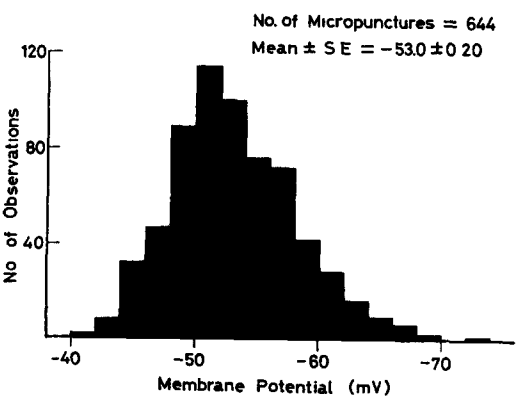

Fig 3. Distribution of the mucosal membrane potentials in epithelial cells of rat duodenum.

($n = 9$) The measured PD_t is, therefore, equal to the calculated PD_t as reported by Gilles-Baillien and Schoffeniels [6], White and Armstrong [8], and Lyon and Sheerin [2]

Effect of variation of $[Cl^-]_m$ *at constant* $[K^+]_m$ *and* $[Na^+]_m$

In order to observe the effects of reducing the Cl^- concentration in the mucosal fluid ($[Cl^-]_m$) on the membrane potential, two test solutions were employed in which Cl^- in the control medium was partially replaced by SO_4^{2-} and the appropriate amount of mannitol was added to keep the total tonicity constant In these solutions, the concentrations of K^+ ($[K^+]_m$) and Na^+ ($[Na^+]_m$) were maintained at 4 2 and 143 0 mM, respectively The mean membrane potential in saline containing 5 5 mM Cl^- was $-57\ 3 \pm 1\ 14$ mV ($n = 22$) and that in saline containing 69 0 mM Cl^- was $-55\ 1 \pm 1.35$ mV ($n = 25$) The difference between the average V_m values in the control medium and in the low Cl^- media has statistically no significance ($P > 0\ 25$) As these potentials were measured several minutes after replacement of solutions, it was assumed that re-equilibration for Cl^- would have been attained between the intracellular and mucosal fluids across the cell membrane. It may be said, therefore, that Cl^- made practically no significant contribution to the membrane potential at least in the steady state This observation is in good accord with the description by Rose and Schultz [7], and Barry and Eggenton [3]

Effect of changing $[K^+]_m$ *at constant* $[Na^+]_m$

Effects of K^+ on the potential profiles were studied with solutions of various K^+ concentrations and constant Na^+ concentration Under these conditions, it was found that as $[K^+]_m$ increased, V_m decreased (depolarization) together with the increase in the PD_t values, as shown in Fig 4. Such a decrease in V_m caused by an increase in $[K^+]_m$ at constant $[Na^+]_m$ has been reported by Barry and Eggenton [3], but the nature of changes in V_m was considerably different A change in V_m caused

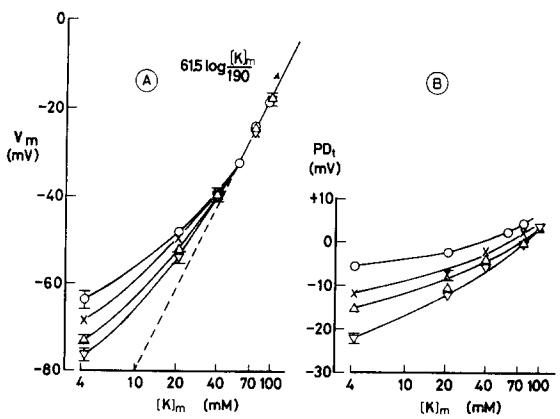

Fig 4 Effect on potential profiles of changing $[K^+]_m$ at constant $[Na^+]_m$ A, V_m values Each point represents the mean of 5–22 observations B, PD_t values Each point represents the mean of 3–6 observations (from 3–6 tissues) ○, ×, △ and ▽ represent the mean potentials observed at constant $[Na^+]_m$ = 71 5, 40 9, 20 4 and 10 2 mM, respectively Vertical bars, standard errors

by increasing $[K^+]_m$ obeyed the Nernst equation expressed by the equation below in the range of $[K^+]_m$ higher than about 60 mM, but deviated from this equation at the lower K^+ concentrations as shown in Fig. 4A.

$$E = -\frac{RT}{F} \ln \frac{[K^+]_m}{[K^+]_i} = -61.5 \cdot \log \frac{[K^+]_m}{190} \quad (1)$$

The value of 190 mM for $[K^+]_i$ thus obtained under high $[K^+]_m$ conditions was in complete accord with the value measured by flame photometry (Okada, Y., Irimajiri, A. and Inouye, A., in preparation). Such behavior of membrane as a potassium electrode in high-K^+ media has been found in many electrically excitable membranes [12–16] and in several inexcitable membranes [17, 18].

The transmural potential changes caused by varying $[K^+]_m$ at constant $[Na^+]_m$ were smaller than the V_m changes, as shown in Fig. 4B. Since the PD_t value could be expressed as the algebraic difference between V_m and V_s, this result suggests that the V_s value also changed by varying $[K^+]_m$, which is contrary to Barry and Eggenton's observation [3].

Effect of changing $[Na^+]_m$ at constant $[K^+]_m$

In order to observe the effects of Na^+ on V_m, various test solutions were employed in which Na^+ concentration was reduced at constant K^+ concentration Under these conditions, it was found that, as $[Na^+]_m$ decreased, V_m was increased (hyperpolarization) together with a decrease in the PD_t values at the low-$[K^+]_m$ region (Fig. 5). A similar dependence of V_m on $[Na^+]_m$ has been reported by McKenney [1], Rose and Schultz [7] and Barry and Eggenton [3]. In the high $[K^+]_m$ region, however, V_m was not affected by a change in $[Na^+]_m$ In contrast to the report of Barry and Eggenton [3], a change in $[Na^+]_m$ did not give linear relationships between V_m and $\log[Na^+]_m$.

The magnitude of changes in PD_t with varying $\log[K^+]_m$ appears to be similar

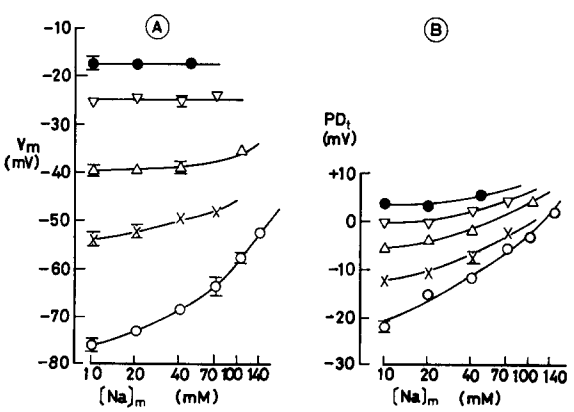

Fig 5 Effect on potential profiles of changing $[Na^+]_m$ at constant $[K^+]_m$. A, V_m values Each point represents the mean of 5–644 observations B, PD_t values. Each point represents the mean of 3–132 observations (from 3–74 tissues). ●, ▽, △, × and ○ represent the mean potentials observed at constant $[K^+]_m$ = 99 5, 75.7, 39 9, 20.7 and 4 2 mM, respectively Vertical bars, standard errors

to that with $\log[Na^+]_m$ (Figs 4B and 5B). Such a finding may be interpreted by nearly equal transmural permeabilities of K^+ and Na^+, as reported by Wright [4] and Frizzell and Schultz [20] But the change in V_m with $\log[K^+]_m$ was far greater than that with varying $\log[Na^+]_m$ (Figs 4A and 5A). This observation strongly suggests that the transmembrane permeability of K^+ is much greater than that of Na^+.

DISCUSSION

The values of membrane potential obtained in this experiment were much greater than the values reported previously in rat jejunum and ileum [1–3], in hamster jejunum [4], and in tortoise small intestine [4–6]. They are also a little higher than the values measured in rabbit ileum [7] and goldfish intestine [9]. The localization of the electrode tips was checked histologically using Mitarai's method [19]. After electrophoresis, all the spot stain of the carmine lithium injected was clearly visible in epithelial cells in the serial histological sections (7 μm). The potentials measured in our experiments, therefore, are undoubtedly the membrane potentials in the epithelial cells Since one of the present authors also obtained such high V_m values in rat jejunum (-54.9 ± 0.9 mV, $n = 34$) and ileum (-54.3 ± 0.1 mV, $n = 30$) (Okada, Y., unpublished observations), these do not seem to be peculiar to the duodenum. In our experience, the low membrane potentials are observed when the isolated mucosa is suspended in the medium in a Petri dish for several minutes before being mounted, or when the serosal musculature is stripped off mechanically Thus one of the most important technical factors for obtaining a high membrane potential is to avoid any mechanical damage, and to maintain the normal blood supply intact until the tissue is mounted.

The transmural potential difference (PD_t) has been analyzed in terms of the equivalent circuit model [7, 8, 20], and Fig. 6 shows one such model According to this model

$$V_m - V_s = PD_t = \frac{R_L}{R} \cdot (E_s - E_m) + \frac{(\mu+1)R_m}{R} E_L \tag{2}$$

$$E \equiv \frac{\mu E_m + E_s}{\mu+1} = \frac{1}{\mu+1} PD_t - V_m \tag{3}$$

where $R = R_m + R_s + R_L$, $R_s/R_m = \mu$ and μ is constant. Since the mucosal and serosal bathing fluids used in the present study were not identical, a significant diffusion potential, E_L, will have been generated across the transmural extracellular pathway [20]. Such a polarity of the epithelial cell, as represented by $(E_s - E_m)$, may be derived from the difference between the permeability to ions of the mucosal and serosal membranes, and/or the electrogenic sodium pump located on the serosal membrane [2–6, 22, 23]. It is difficult to estimate changes in E_m and in E_s separately, therefore we adopted the weighted mean of E_m and E_s, i.e $(\mu E_m + E_s)/(\mu+1)$, as a parameter of the effective e m f of the epithelial cell membrane. When $\mu \gg 1$, we can safely put $E = -V_m$. This approximation seems to be plausible because of the reduction in the electrical resistance of the mucosal membrane caused by the presence of microvilli.

As the intestinal epithelium can be classified as a leaky tissue that has a low-resistance, transmural, extracellular pathway [7, 20, 21], i e. R_L is far smaller than R,

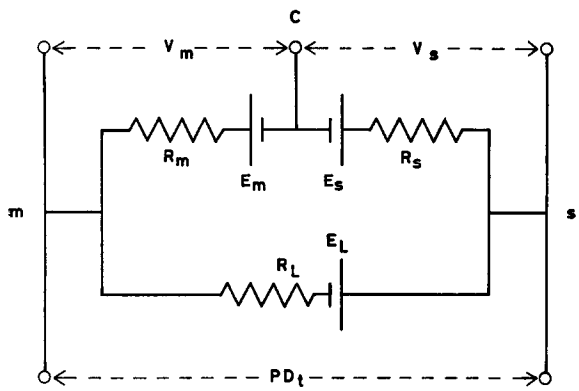

Fig 6 An equivalent electrical circuit. R_m and R_s are the resistances of the mucosal and serosal membranes, respectively R_L represents a transepithelial shunt resistance. E_m and E_s are the e.m.f. values for the mucosal and serosal membranes, respectively E_L is the diffusion potential through the transepithelial shunt pathway.

PD_t would be dominated by E_L. It is generally accepted that the transmural permeabilities of K^+ and Na^+ are nearly equal in the intestinal epithelium [4, 20]. Indeed, as shown in Fig. 7a, a single curvilinear relationship of PD_t to $\log([K^+]_m+[Na^+]_m)$ approximately applies, irrespective of the value of $[K^+]_m/[Na^+]_m$. Slight differences between the points of high $[K^+]_m$ and those of low $[K^+]_m$ could be explained by a slight difference between the permeability coefficients for K^+ and Na^+ (P_K, P_{Na}) through the shunt pathway [4, 20]. Moreover, the slope of the curve in the high salt concentration range is around 50 mV per 10-fold change in the salt concentration, and this value is in fairly good agreement with the theoretical value (around 60 mV) when

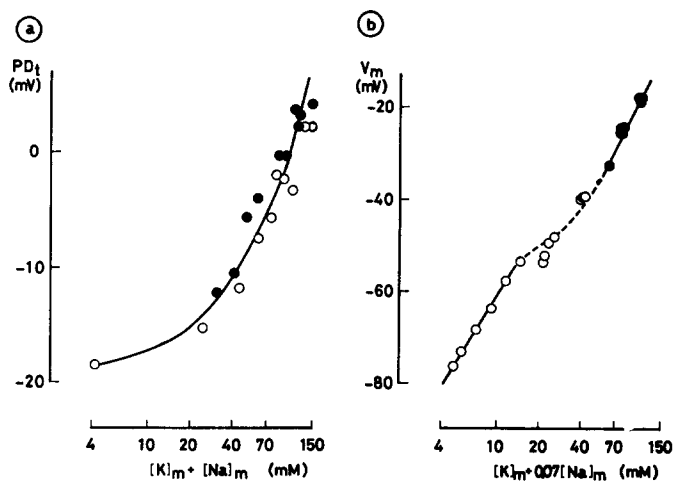

Fig. 7. Relationship between PD_t or V_m and cation concentration ($[K^+]_m+\alpha \cdot [Na^+]_m$) a, PD_t and ($[K^+]_m+\alpha \cdot [Na^+]_m$) where $\alpha = 1\,0$. ○, $[K^+]_m < [Na^+]_m$, ●, $[K^+]_m > [Na^+]_m$. Each point represents the mean of 3–132 observations with the standard error less than ± 1 mV. b, V_m and ($[K^+]_m + \alpha$ $[Na^+]_m$) where $\alpha = 0.07$. ○, $[K^+]_m < 60$ mM; ●, $[K^+]_m > 60$ mM. Each point represents the mean of 5–644 observations with the standard error less than ± 3 mV

it is corrected by dividing by the partial ionic shunt conductance (e g. 85 % in rabbit ileum [20]). The fact that the slope becomes smaller in the low salt concentration range might be attributed to increases in R_L due to decreasing Na^+ and K^+ and increasing $Tris^+$ Thus it might be conceded that PD_t is dominated by E_L and approximately expressed as a function of $([K^+]_m+[Na^+]_m)$

On the other hand, the values of V_m observed were remarkably affected by a change in $[K^+]_m$. In the range of $[K^+]_m$ higher than 60 mM, a change in V_m obeyed the Nernst equation as stated above Since the Cl^- made no significant contribution to V_m, at least in the steady state, we could safely assume that the constant-field equation [24, 25] for the effective e.m.f , E, of epithelial cell membrane can be reduced to the following form by neglecting the term of the Cl^- concentration provided that the extra- and intracellular activity coefficients are assumed to be nearly identical.

$$E = -61.5 \cdot \log \frac{[K^+]_m+\alpha \cdot [Na^+]_m}{[K^+]_i+\alpha \ [Na^+]_i} \tag{4}$$

where α is the ratio of the permeability coefficients (P_{Na}/P_K) Our finding that V_m obeyed the Nernst equation for a reversible K^+ electrode in the range of $[K^+]_m =$ 60 to $\sim$ 100 mM, strongly suggests that $E_m = -V_m$, in this range of $[K^+]_m$ at least, and that $1/(\mu+1)$ is quite small In this region, therefore, it seems very likely that the contribution of E_L to V_m is negligible, and α is nearly equal to zero In general, however, a change in E in response to a change in $[K^+]_m$ or $[Na^+]_m$ cannot be estimated from a change in V_m alone, as seen from Eqn 3. To estimate it semiquantitatively, we attempted application of the following approximation to Eqn 4. In the present experiments, $[Na^+]_i$ and $[K^+]_i$ remain unknown, but they are expected to vary with some relationship to $[Na^+]_m$ and $[K^+]_m$. As the first approximation, we assumed that E can be written as.

$$E = f(x),\ x \equiv [K^+]_m+\alpha \ [Na^+]_m \tag{5}$$

where $f(x)$ represents a function of x For instance, putting $\alpha = 0\,07$, a fairly good correlation of V_m with $\log([K^+]_m+\alpha \cdot [Na^+]_m)$ was found as shown in Fig 7b. It should be noted here that the slopes are about 50 and 60 mV for the low-$[K^+]_m$ region and high-$[K^+]_m$ region, respectively As α $[Na^+]_m$ is negligible compared with $[K^+]_m$ in the range of x higher than 60 mM for $\alpha < 0\,1$, it is quite natural from the results presented in Fig 5A, that the Nernst slope should be obtained in this region Such a correlation between V_m and x as seen in Fig. 7b suggests that V_m is largely, if not solely, dependent upon E

We then obtain the following relation provided that Eqn 5 is practically applicable

$$\left(\frac{\partial E}{\partial \log [K^+]_m}\right)_{[Na^+]_m} = \frac{1}{\alpha} \frac{[K^+]_m}{[Na^+]_m} \left(\frac{\partial E}{\partial \log [Na^+]_m}\right)_{[K^+]_m} \tag{6}$$

because

$$\left(\frac{\partial f}{\partial [K^+]_m}\right)_{[Na^+]_m} = \frac{\partial f}{\partial x} = \left(\frac{1}{\alpha} \left(\frac{\partial f}{\partial [Na^+]_m}\right)\right)_{[K^+]_m}$$

From Eqn 3, we can obtain

$$\frac{\partial E}{\partial \log [K^+]_m} = -\frac{\partial V_m}{\partial \log [K^+]_m} + \frac{1}{\mu+1} \cdot \frac{\partial (PD_t)}{\partial \log [K^+]_m}$$
$$\frac{\partial E}{\partial \log [Na^+]_m} = -\frac{\partial V_m}{\partial \log [Na^+]_m} + \frac{1}{\mu+1} \cdot \frac{\partial (PD_t)}{\partial \log [Na^+]_m} \quad (7)$$

When the value of μ is given, the value of each term in the right hand of these equations can be obtained from the slopes in Figs 4 and 5. The α values can be estimated from Eqn 6 by substituting $\partial E/(\partial \log[K^+]_m)$ and $\partial E/(\partial \log[Na^+]_m)$ with the values thus obtained in the range of $[K^+]_m$ lower than 60 mM. The α values estimated with $\mu = 1$ are plotted in Fig. 8. It shows that α values increase abruptly from 0 to around 0.07 at $[K^+]_m = 30$ to ~ 50 mM, an observation corresponding to the inflection at intermediate x-values in Fig. 7b. It is apparent from Figs 4A and 5A that the α vs $\log[K^+]_m$ relationship for $\mu \gg 1$ (i.e. $E = -V_m$), is quite similar to that illustrated in Fig. 8, the only difference being that the α value at $[K^+]_m < 30$ mM is a little greater (around 0.13). Moreover, for any μ values between 1 and 10, the pattern of the α vs $\log[K^+]_m$ relationship is quite similar. Such a result seems to suggest an abrupt change in membrane properties with the change in external K^+ concentrations. A similar result has already been reported in HeLa cell membrane [18]. The above reasoning is based on the approximation of E in the form given in Eqn 5. Our recent flame photometric determination of $[Na^+]_i$ and $[K^+]_i$ on rat duodenum (Okada, Y., Irimajiri, A and Inouye, A , in preparation) showed that the following relationship applied practically in the range of $[K^+]_m$ lower than about 70 mM·

$$[K^+]_i + a \cdot [Na^+]_i = [K^+]_m + a \cdot [Na^+]_m + \text{constant} \quad (8)$$

where a is a constant far smaller than 1 (0 to ~ 0.2). Since α is far smaller than 1, as seen in Fig. 8, the use of Eqn 5 might be justified at least for a semiquantitative estimation of α.

Many investigators [2–6, 22, 23] suggested that the electrogenic sodium pump

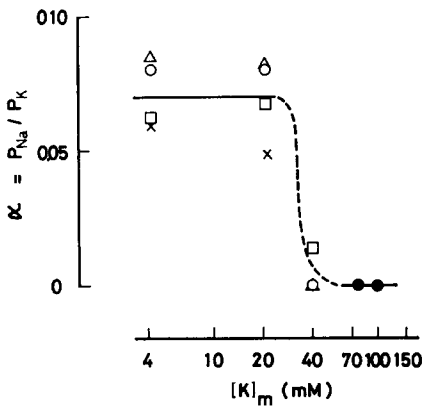

Fig. 8. Relationship between α ($= P_{Na}/P_K$) values and $\log[K^+]_m$. ○, △, □ and × represent α values estimated with $\mu = 1$ by applying Eqns 6 and 7 in the range of $[K^+]_m$ lower than 60 mM and constant $[Na^+]_m = 10.2$, 20 4, 40.4 and 71 5 mM, respectively. ●, α values estimated by Eqn 1 at higher $[K^+]_m$

is present on the serosal membrane in the intestinal epithelial cell If the electrogenic sodium pump made a significant contribution to the membrane potential, Eqn 4 would not be applicable. Mullins and Noda [26] showed that the Goldman equation could be applicable if α is substituted by α/r (where r is the coupling ratio of Na^+ efflux and K^+ efflux), when the electrogenic sodium pump is acting in the stationary state. Under the present experimental conditions, therefore, the aforegoing discussion would be acceptable, even if the electrogenic sodium pump contributed significantly to the membrane potential. In this case, however, the estimated α values do not represent the $P_{Na} : P_K$ ratio straightforwardly, and the contribution of r should be taken into account

The aforegoing discussion is based on the assumption that not only the activity coefficients of Na^+ and K^+ in bathing solutions but also those within the cell are identical and therefore cancel out in the Goldman equation. Many studies [27–33] have shown that the activity coefficient of Na^+ within the cell is smaller than in the bathing fluid, and a recent experimental study [34] on the epithelial cells of bullfrog small intestine has shown that the activity coefficient of Na^+ within the cell is 0.5, whereas that of K^+ is 1.0 Nevertheless, we found that our qualitative conclusions were not altered even when the values of the activity coefficients cited above were applied

ACKNOWLEDGEMENTS

Thanks are due to Dr A Irimajiri for pertinent discussion and Miss M. Ohara for assistance with the manuscript. The authors are indebted to Mr K. Yamato for technical assistance

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